The University of Chicago
FOUNDED BY JOHN D. ROCKEFELLER

THE EARLY DEVELOPMENT OF MARINE ANNELIDS

A DISSERTATION SUBMITTED TO THE FACULTIES OF THE GRADUATE SCHOOLS OF ARTS, LITERATURE, AND SCIENCE, IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

BY
ALBERT D. MEAD

[Reprinted from JOURNAL OF MORPHOLOGY, Vol. XIII, No. 2]

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THE EARLY DEVELOPMENT OF MARINE ANNELIDS.

A. D. MEAD.

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THE material for the preparation of this paper was procured at Woods Holl, Mass. The work has been carried on at the Marine Biological Laboratory, at Clark University, and at the University of Chicago under the direction of Dr. C. O. Whitman. I desire here to express my appreciation of the generous treatment which I have received at these institutions, and especially to thank Dr. Whitman for his interest and friendly counsel, which encouraged me to pursue these researches.

PART I.—DESCRIPTIVE.

A. AMPHITRITE ORNATA VERRILL.

Colonies of *Amphitrite* are found in several places in the vicinity of Woods Holl, Mass., being more abundant at Lackey's Bay, Hadley Harbor, and Ram Island. The animals live in tough mud-tubes, from just below low-water mark to a considerable depth. A little experience enables one to locate the tubes easily, for every one has two openings, each at the summit of a peculiar mound which resembles a small anthill. The capture of the worms themselves is attended, however, with a good deal of difficulty, since they retreat to a considerable depth beneath the surface of the mud. The limits of the breeding season are unknown. Although about eight hundred worms were collected, in lots of twenty or thirty, between the first of June and the last of August, only seldom were ripe eggs and ripe spermatozoa obtained. Naturally the eggs are not found in the sea, for they are just the color of the mud, and are doubtless discharged free into the water, and soon scattered by the currents.

The females are darker colored than the males, and the eggs can often be seen through the body wall. It is useless to cut the animals open, for, if the sexual products are mature, they will be discharged, usually at about 6 o'clock in the evening, more often on the day of capture, sometimes the next day. The rarity of ripe specimens is partly compensated for by the enormous number of eggs which may be obtained from one female. They may be kept in the sea-water for an hour or more without harm, and then successfully fertilized by simply adding the sperm, though it is necessary to rinse the eggs later to rid them of the superfluous spermatozoa. The late larvæ, five days old or more, are best kept in a dish with fresh ulva ; the trochophores soon lose their cilia and creep upon the sea-weed.

For killing, I have used Kleinenberg's picro-sulphuric (strong), chrom-formic, Perenyi's fluid (for surface views), and Flemming's fluid, weaker formula.

My first material was so damaged by the tannin which the alcohol extracted from the cork stoppers that I discarded the

latter and used plugs of absorbent cotton covered with thin linen paper to prevent losing the minute eggs in the cotton. The vials were then packed in glass jars with labels toward the outside, and the whole jar filled with strong alcohol.

In staining for surface views I have always obtained the best results with Delafield's hæmatoxylin, especially with Conklin's method of using the stain diluted and acidulated. The stained eggs are cleared in clove oil or cedar-wood oil in the ordinary way, and mounted in balsam, the cover glass being supported by four paper "feet." A little clove oil allowed to mix with the balsam keeps the latter soft, so that for weeks the egg can be rotated and drawn with the camera, in any position.

In drawing surface views with the camera lucida, when the cell outlines are faint and complicated, I have used thin black paper and a soft lead pencil, the point of which has been painted with *chinese white.* The advantages of this method are twofold; the image of the whitened pencil-point and that of the egg are both more distinct, and the pencil lines on the paper are never confused with the lines of the image. By simply smearing the back of the paper with soft lead and stub, the sketch may be easily transferred to drawing paper.

In the belief that it is unwise to depart from a nomenclature which involves nearly all the observations in this particular type of cleavage, unless a greatly superior one can be substituted, I have adopted that introduced by Wilson in his *Nereis* paper,[1] and employed with certain modifications by Heymons[2] and Lillie[3] (Kofoid's[4] ingenious system of naming the cells avoids possible objections to Wilson's system, but is fraught with certain practical disadvantages). I have, however, amended Wilson's scheme in the following points to adapt it to comparative description.*

[1] Wilson, E. B.: The Cell Lineage of Nereis. *Journ. of Morph.*, vol. VI, 1892.

[2] Heymons: Zur Entwicklungsgeschichte von Umbrella mediterranea. *Zeitschr. f. wiss. Zool.* Bd. 56, 1893.

[3] Lillie, F. R.: The Embryology of the Unionidae, A Study in Cell Lineage. *Journ. of Morph.*, vol. X, no. 1, 1895.

[4] Kofoid: On Some Laws of Cleavage in Limax. *Proc. Amer. Acad. Arts and Sci.*, January, 1894.

* Some of the points have been mentioned by Kofoid and Lillie.

(1) Instead of designating cells of *several successive generations* by the same letters, *e.g.*, the "macromeres" *A*, *B*, *C*, *D*, I have used subscripts, thus : A, A_1, A_2, etc. (2) Instead of naming the daughter cells sometimes from their relative position and sometimes from their relative size, I have named them uniformly with respect to their relative *position*, giving the cell

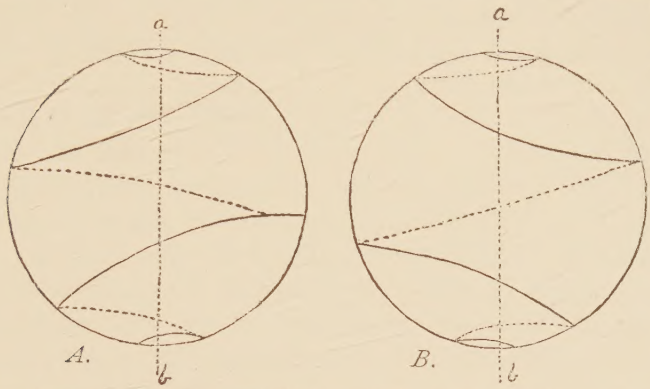

FIG. I. — Diagrams of egg with circumscribed loxodromic curves. *a–b*, axis. The curve in *A* represents the general direction of the cleavage furrows in *right oblique cleavage ;* in *B* that of the furrows in *left oblique cleavage*.

nearer the vegetative pole the larger exponent. For example, a^2 divides into $a^{2.1}$ and $a^{2.2}$, and the latter is nearer the vegetative pole.

This nomenclature is employed purely as an instrument of convenience, and I do not thereby commit myself to any implied theory of development.

When the cleavage plane of a dividing cell takes the general direction of a loxodromic curve described about the egg in the direction indicated in text Fig. I, *A*, the cleavage is designated as *right oblique ;* when in the direction of the curve in Fig. I, *B*, as *left oblique*. Similarly, we may speak of vertical and horizontal cleavages, when the furrow takes the direction of the meridian, or one at right angles to it.

I. CLEAVAGE TO 64 CELLS.

The normal fertilized egg of *Amphitrite* is nearly spherical and about 100μ in diameter, although there is considerable individual variation. It is enclosed in a much-wrinkled mem-

brane, and is perfectly opaque. The cleavage nucleus is slightly eccentric, lying nearer the polar globules, and is surrounded by a thick layer of protoplasm, while the yolk is mostly segregated at the vegetative pole. For descriptive purposes the position of the polar bodies indicates approximately the *animal pole* of the egg and the center of the *anterior hemisphere;* the point 180° from this is the *vegetative pole*, the center of the *posterior hemisphere;* the line joining the two points is the vertical axis of the egg.

When the first cleavage spindle is formed, the egg is slightly oval, and the spindle always parallel with the long axis, on the same level as the segmentation nucleus, and *nearer one end* of the egg (Fig. 1, Pl. X). At this time the egg is completely oriented with reference to the future cleavage furrows. The rate of cleavage is influenced directly by the temperature; in July the first polar globule appears in less than half an hour after fertilization, and the first cleavage furrow about thirty minutes later.

The first furrow is meridional (parallel with vertical axis), and appears all round the egg at about the same time, though it sinks in somewhat more rapidly on the anterior hemisphere. It divides the egg into two blastomeres of unequal size, *A–B* and *C–D* (Fig. 2), and in a few minutes these divide nearly simultaneously, though the larger (*C–D*) is sometimes a little in advance. The direction of the cleavage is always *left oblique;* the karyokinetic spindles, even when in the equatorial-plate stage, are inclined to the plane of the equator, and indicate the direction of the cleavage (Fig. 3). Consequently the cells *B* and *D* in the 4-cell stage lie somewhat lower (nearer the vegetative pole) than *A* and *C*, and meet in a "cross-furrow" (Figs. 4, 5); but the "precocious formation" of the cross-furrow found in some eggs, for example, in *Crepidula* and *Nereis*, is not apparent in *Amphitrite*. In the cleavage of the two blastomeres, as in the first cleavage, the furrows sink in more rapidly on the anterior hemisphere than on the yolk-laden posterior hemisphere (Fig. 4).

The cell *D* of the 4-cell stage (Figs. 4–6) is considerably larger than the other cells, and its descendants play a conspicu-

ous rôle in the development. Neither of the cleavage furrows coincides with the sagittal plane of the future embryo; this plane passes through the cells *B* and *D* (cf. Wilson,[1] p. 386).

All four cells undergo an almost synchronous right oblique cleavage, and an 8-cell stage results (Figs. 5, 6, 7). In consequence of the obliquity of the cleavage, the upper cells a_1, b_1, c_1, d_1 are not directly superimposed on the large ones A_1, B_1, C_1, D_1, but alternate with them. For convenience in description the cells a_1, b_1, c_1, d_1, or their descendants, will be regarded as constituting the *upper* or *anterior hemisphere*.

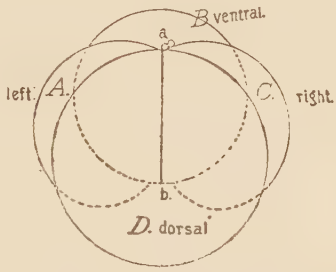

FIG. II. — Diagram of 4-cell stage. *a*, animal pole; *b*, vegetative pole; *A*, *B*, *C*, and *D*, first four blastomeres, the anlagen respectively of the left, ventral, right, and dorsal quadrants of the trochophore.

The relative position of the upper and the lower quartette of cells is significant, for it alters the direction of the second cleavage furrow, twisting it so that its direction on the upper hemisphere is different from that on the lower, — a relation which is maintained throughout the subsequent cleavage. Each of the four cells has divided unequally, but with a remarkable result; on the posterior hemisphere in the 8-cell stage, D_1 is larger than A_1, B_1, C_1, which latter are about equal to one another in size, while on the anterior hemisphere, d_1 and c_1 are equal in size, but larger than a_1 and b_1. The four original cells have undergone a sort of *compensating division*, which establishes upon the *anterior hemisphere* a bilaterally symmetrical group of cells, whose plane of symmetry nearly coincides with the second cleavage furrow (Fig. 7).

The polar globules remain where they were formed, and lie near the meeting-point of the four upper blastomeres. There is already a small segmentation cavity (Fig. 7). The yolk is more abundant in the four lower cells, but the egg follows the same course of development whether in one position or another, and often has been observed to develop with the vegetative pole uppermost.

8–16 cells. — Except for slight variation, the eight cells cleave synchronously, and always obliquely to the left (Figs.

8–10). The sixteen cells (nearly ready to divide again) are seen in Figs. 11–14. In consequence of the regularity of the last cleavage, they are arranged in four alternating zones of four cells each. The cells of the second zone on the upper hemisphere, $a^{1.1}$, $b^{1.1}$, $c^{1.1}$, $d^{1.1}$, are the parent cells of the *primary prototroch* — "trochoblasts" (Wilson), so I shall call them *primary trochoblasts*. In *Amphitrite* these cells are more strictly "trochoblasts" than in *Nereis*, for *all their descendants* become prototrochal cells, while in *Nereis*, according to Wilson, some do not. The egg at this stage has a segmentation cavity of considerable size.

As we shall see below, certain cells of the lower hemisphere, a^2, b^2, c^2, also contribute to the prototroch in *Amphitrite*. d^2 (X), however, does not do so, but has a special destiny: it is to form the whole ectoderm of the trunk, including proctodœal cells and paratroch. Therefore I shall call it, after Wilson, the *somatoblast*.

16–32 cells, Figs. 11–16. — All sixteen cells soon cleave *obliquely to the right*, though not quite synchronously. The two largest cells, d^2 and D_2, divide first (Fig. 14); the cells of the anterior hemisphere next (or frequently at the same time); the remaining cells of the lower hemisphere last. Notwithstanding these slight time variations, a 32-cell stage is attained, which may be described as consisting of eight quartettes of cells alternating with one another from pole to pole (Fig. 16). The thirty-two cells are arranged as follows: $a_{1.2}$, $b_{1.2}$, $c_{1.2}$, and $d_{1.2}$ meet at the animal pole; in the outer angles between them lie $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, and $d^{1.2}$; alternating with these are four of the daughter cells of the trochoblasts, $a^{1.1.1}$–$d^{1.1.1}$; and next the remaining four, $a^{1.1.2}$–$d^{1.1.2}$. These are all the cells of the *anterior hemisphere*. Alternating with the last four cells, come $a^{2.1}$, $b^{2.1}$, $c^{2.1}$, and $d^{2.1}$ (= X), which, with the exception of $d^{2.1}$, contribute nearly all their substance to the completion of the prototroch, and may be called *secondary trochoblasts*. Next in order are the quartettes, $a^{2.2}$–$d^{2.2}$, a^3–d^3, and at the lower pole, A_3–D_3.

Up to this point, in the transition from one stage to another, all the cells divide almost simultaneously and in the same direction; all the divisions are oblique, and the direction — to the right or left — alternates with each succeeding stage

(cf. "Law of Alternating Spiral Cleavage," Wilson,[1] Kofoid,[4] etc.). If the cleavage were to continue in this regular fashion, we should find each of the thirty-two cells dividing at about the same time, and the cleavage furrow cutting each cell obliquely to the left. The result would be sixty-four cells having the regular arrangement characteristic of the earlier stages. *Amphitrite* falls slightly short of the ideal, for some cells divide sooner than others, and the most precocious are ready to divide again, when the most tardy ones have divided once. This tendency was recognized in the earlier cleavage, 16–32 cells, and has now become accentuated (Pl. XI, Figs. 17–28). Except for this comparatively slight irregularity in time of division, the cleavage of all thirty-two cells takes place with the rhythmical regularity characteristic of the typical alternating oblique cleavage: the cleavage of every cell is oblique and to the left.

The transition from the 32 to the 64-cell stage is especially interesting, because it is the last instance of the rhythmical division of all the cells, and because by these divisions the prospective germ layers become completely segregated into special cells, and the primary prototroch differentiated. The order of division can be seen by consulting Figs. 17–28. As a rule, almost immediately after the completion of the 32-cell stage, D_3 divides into D_4 and d^4 ($= M$). The former is the smaller and is pushed outward in a peculiar manner so that it lies at first above the level of the other cells (Fig. 20), but later crowds in among them, and forms part of the entoderm plate. d^4 ($= M$) is the original *mesoderm cell*, and apparently gives rise to all the mesoderm of the body. Before this division is completed, the eight daughter cells of the *primary trochoblasts* prepare to divide (Figs. 17, 21, 22, colored brown). All the sixteen cells resulting from this cleavage acquire cilia, and constitute the *primary prototroch* (Figs. 18, 19). Meanwhile the four animal-pole cells divide almost simultaneously, but with many slight variations in different eggs. The innermost products, $a_{1.3}$, $b_{1.3}$, $c_{1.3}$, and $d_{1.3}$, constitute the "*apical rosette*," while the outermost are parent cells of another characteristic pattern, — the "*cross*" $a^{1.3}$, $b^{1.3}$, $c^{1.3}$, and $d^{1.3}$ (colored blue, Figs. 18, 24).

The two daughter cells of the somatoblast, *i.e.*, $d^{2.1}$ and $d^{2.2}$

(X_2 and x^2), are among the first to divide (Fig. 20, also blue). Hereafter I shall speak of these cells or their descendants as the *somatic-plate*, for they give rise in *Amphitrite* not only to the *ventral plate* of the trunk, but to the *lateral* and *dorsal* areas as well. The secondary trochoblasts also divide early: $a^{2.1}$, $b^{2.1}$, and $c^{2.1}$ (Figs. 21, 22).

Soon after the cleavage of the rest of the cells takes place. A_3, B_3, and C_3, whose products, A_4, B_4, C_4 and a^4, b^4, c^4, with those of D_4, give rise to the entoderm (stippled in figures), are among the last to divide.

It is essential to obtain a clear idea of the orientation at the ideal 64-cell stage, for with reference to it the future cleavage will be described, one group of cells at a time. In the following table the cells are arranged in groups from the animal to the vegetative pole:

ANTERIOR HEMISPHERE	4	$a_{1.3}$ $b_{1.3}$ $c_{1.3}$ $d_{1.3}$	rosette	Ectoderm
	4	$a^{1.3}$ $b^{1.3}$ $c^{1.3}$ $d^{1.3}$	cross	
	8	$a^{1.2.1}$ $b^{1.2.1}$ $c^{1.2.1}$ $d^{1.2.1}$ $a^{1.2.2}$ $b^{1.2.2}$ $c^{1.2.2}$ $d^{1.2.2}$	intermediate cells	
	16	$a_1^{1.1.1}$ $b_1^{1.1.1}$ $c_1^{1.1.1}$ $d_1^{1.1.1}$ or ap^1–dp^1 $a_2^{1.1.1}$ $b_2^{1.1.1}$ $c_2^{1.1.1}$ $d_2^{1.1.1}$ ap^2–dp^2 $a_1^{1.1.2}$ $b_1^{1.1.2}$ $c_1^{1.1.2}$ $d_1^{1.1.2}$ ap^3–dp^3 $a_2^{1.1.2}$ $b_2^{1.1.2}$ $c_2^{1.1.2}$ $d_2^{1.1.2}$ ap^4–dp^4	primary prototroch	
EQUATOR				
POSTERIOR HEMISPHERE	8	$a^{2.1.1}$ $b^{2.1.1}$ $c^{2.1.1}$ $a^{2.1.2}$ $b^{2.1.2}$ $c^{2.1.2}$	secondary prot. cells	
		$d^{2.1.1}$ $d^{2.1.2}$	somatic-plate cells	
	8	$d^{2.2.1}$ $d^{2.2.2}$		
		$a^{2.2.1}$ $b^{2.2.1}$ $c^{2.2.1}$ $a^{2.2.2}$ $b^{2.2.2}$ $c^{2.2.2}$	ectoderm of lower hemisphere	
	8	$a^{3.1}$ $b^{3.1}$ $c^{3.1}$ $d^{3.1}$ $a^{3.1}$ $b^{3.1}$ $c^{3.1}$ $d^{3.1}$		
		d^4	mesoderm	Mesoderm
	8	a^4 b^4 c^4 A_4 B_4 C_4 D_4	entoderm cells	Entoderm
	64			

Summary.— The fertilized egg of *Amphitrite* is spherical, about 100μ in diameter, and covered with a wrinkled membrane. The first cleavage is unequal, and in the 4-cell stage one blastomere is larger than any of the others. A vertical plane passed through the larger blastomere and the one diagonally opposite corresponds to the sagittal plane of the future embryo. The number of cells increases in geometrical progression from one to sixty-four. From the 2 to the 64-cell stage every cleavage furrow is oblique to the meridian of the egg, and the direction of the obliquity regularly alternates with each succeeding cleavage. From the 8-cell stage onward we distinguish an anterior or upper hemisphere — the four upper cells or their descendants, and a posterior or lower hemisphere — the four lower cells or their descendants. In consequence of a slight rotation of one hemisphere upon the other, the second cleavage furrow more nearly coincides with the future sagittal plane in the upper than in the lower hemisphere. In the 64-cell stage the material for the several germ layers is completely sorted out so that one cell represents the future mesoderm, seven cells the entoderm, and the remaining fifty-six cells, the ectoderm. The latter fall naturally into groups, which from the animal pole are: (1) rosette, (2) cross, (3) intermediate cells, (4) primary prototroch, (5) secondary prototroch, (6) somatic-plate, (7) the rest of the ectoderm of the lower hemisphere. Then come the mesoderm and entoderm. The regularity of the cleavage ceases abruptly at the 64-cell stage, so that from this time one can best follow the cells in groups.

II. Later Cleavage to Formation of Paratroch.

a. *Anterior hemisphere.*

By the time the 64-cell stage is actually attained, the parent cells of the cross, $a^{1.3}$, $b^{1.3}$, $c^{1.3}$, $d^{1.3}$, contain karyokinetic spindles and soon divide, sometimes simultaneously, but often with slight differences in time (Fig. 24). The furrows always cut the meridian of the egg at right angles. *There is never a trace of the oblique cleavage characteristic of all the previous divisions.* The resulting cells form the pattern of a cross, with arms in-

clined at an angle of 45° to the median plane, while the *rosette* lies in the middle of the cross (Figs. 18, 24, 29, cross, blue). The rosette cells later divide obliquely to the right, thus continuing in the alternating cleavage (Fig. 30). The intermediate cells, those in the angles between the arms of the cross, all divide in the same direction — right oblique — though not all at the same time. As a general rule, the larger ones divide first, the smaller last (Fig. 30).

The sixteen cells of the primary prototroch *never divide again*, but are flattened down so as to present a very even surface, and soon become covered with cilia.

The succeeding divisions of the cross cells are of great interest. They are exactly bilateral, so that the divisions on one side are the mirrored image of those on the other (Figs. 30–36). First, the distal cells in the dorsal arms of the cross divide, $c^{1.3.2}$, $d^{1.3.2}$; but, before these divisions are completed, spindles appear in the corresponding cells of the ventral arms, $a^{1.3.2}$, $b^{1.3.2}$, and in the *proximal* cells of the dorsal arms, and soon also in the proximal cells of the ventral arms, $a^{1.3.1}$ and $b^{1.3.1}$. When all these divisions are completed, each arm of the cross has three cells in a row and an extra one at the base. *The cross is not only symmetrical with respect to the median sagittal plane, but nearly so with respect to a plane at right angles to this.* So far, and even farther, the cross in *Amphitrite* is exactly comparable to that in *Nereis* (cf. Wilson,[1] Pl. XVI, Fig. 40). The middle cells in the dorsal arms, corresponding to the "nephroblasts" in *Nereis*, never divide again, and, except for a small area left at the surface, are covered over by the surrounding cells, and become the huge *dorsal umbrellar mucous glands* (Figs. 31–37, text Figs. XI–XVII, *gl.l.* and *gl.r.*, p. 257). The striking similarity between the dorsal and ventral arms of the cross at once suggests that the middle cells in the ventral arms have a destiny similar to that of *gl.r.* and *gl.l.* — Unlike the dorsal cells, they each divide once meridionally, but this I think is their last division (Fig. 33). They are soon partially covered over like the corresponding cells in the dorsal arms. Since two pairs of unicellular mucous glands occupy a position in the later larva exactly corresponding to these two pairs of

cells (*gl.*, text Figs. XI–XVII), it seems extremely probable that the middle cells in the ventral, as well as in the dorsal arms of the cross, give rise to unicellular mucous glands. The outlines of the *cross* are destroyed by the further cleavage of its component cells (Figs. 36, 37). These later divisions are interesting because they are manifestly bilateral, and because they bear a remarkable correspondence to the same divisions in *Nereis*, as far as the latter are figured (*Nereis*,[1] Figs. 41–44).

Of the *intermediate cells* only those between the dorsal arms of the cross have been followed through several generations. My object in following these was to ascertain what cells, if any, migrate from the anterior to the posterior hemisphere, through the mid-dorsal interruption of the prototroch, that is, through the gap between the prototrochal cells of the *d* and *c* quadrants. In the 64-cell stage these cells are two in number, $d^{1.2.1}$ and $d^{1.2.2}$ (Figs. 18, 24, 29). The latter is much larger and divides first, obliquely to the right (Fig. 30), and the former divides in the same direction (Fig. 45, $d^{1.2.1}$).* The posterior product of $d^{1.2.2}$ divides and for the three descendants of $d^{1.2.2}$ we will substitute the letters, $l.^1$, $l.^2$, and $l.^3$ (Figs. 32, 34–36, 45). Next $l.^1$ divides (Fig. 51) and then $l.^3$, making five cells in the group (Fig. 54). By this time the interruption in the prototroch has become much narrowed by the approach of the huge prototrochal cells from either side, and the *l.* group is seen to have taken a position *below the narrowest part* (Figs. 58, 60). Meanwhile the anterior product of $d^{1.2.2}$ divides. One of the daughter cells is extremely minute, has a deeply staining nucleus, and serves as an excellent landmark (asterisk, Figs. 35, 36, 51, 54, 58, 59, 60). When the prototrochal cells have met in the middle line, this minute cell with its twin, which also has a peculiar appearance, lies just above the suture (Fig. 60).

Therefore, returning to Fig. 24 or 29, I think we are warranted in saying that about half the cell $d^{1.2.2}$ and possibly part of $d^{1.2.1}$ contribute to the structures posterior to the pro-

* In the figures, the progeny of $d^{1.2.1}$ are indicated by the heavy outlines of their nuclei, while the posterior product of the division of $d^{1.2.2}$ (and its descendants) are distinguished by *shaded nuclei* (Figs. 34–36).

totroch. It is obvious that, when the prototroch has united dorsally, communication is shut off between the general ectoderm of the two hemispheres.

I have not observed any further divisions of the rosette cells, now eight in number, and believe that they bear the apical tuft of cilia, which appears long before the interruption of the prototroch is obliterated.

b. *Posterior hemisphere.*

Completion of prototroch. — Shortly after the 64-cell stage, the *secondary trochoblasts*, $a^{2.1.1}$, $a^{2.1.2}$, $b^{2.1.1}$, $b^{2.1.2}$, $c^{2.1.1}$, $c^{2.1.2}$, all divide obliquely to the right in accordance with the alternating rhythm (Figs. 26, 27, 28).

These divisions are precisely alike in each of these three quadrants, as shown in text Fig. III. $a^{2.1.1}$, $b^{2.1.1}$, and $c^{2.1.1}$ divide about equally, the others, $a^{2.1.2}$, $b^{2.1.2}$, $c^{2.1.2}$, very unequally. Of the resulting four cells in each group, the three larger ones are the *secondary prototrochal cells*, which never divide again, but soon become covered with cilia and form *part of the prototroch.* By virtue of their position they fill the gaps in the *primary prototroch*, excepting, of course, the mid-dorsal one, which, as we have seen, is filled by the concrescence of the primary prototrochal cells. By the addition of these nine cells, the prototroch is completed, and its twenty-five component cells may all be recognized even after the larva has begun to elongate (Figs. 57, 59, 61–63, and text Figs. V, VI). The outlines can always be seen, though difficult to follow in later stages.

Fig. III. — Diagram showing the relation of the cells in the region of the *secondary trochoblasts* just before the completion of the prototroch. It applies to the *A*, *B*, and *C* quadrants in both *Amphitrite* and *Clymenella*. The lightly stippled cells p^1 p^2, etc., belong to the primary prototroch; the heavily stippled cells (from the secondary trochoblasts) complete the prototroch.

Somatic-plate cells. — In the *posterior quadrant* $d^{2.1.1}$ and $d^{2.1.2}$ ($= X_2$ and x^2) behave in a manner very different from that of

the corresponding cells in the other quadrants. They divide somewhat in advance of the latter, and though $d^{2.1.2}$ ($= x^2$) divides in the same direction as the corresponding cells in the other quadrants, *i.e.*, continues in the alternating rhythm, $d^{2.1.1}$ ($= X_2$) does not, but divides somewhat obliquely in the *other direction* (Fig. 38).

The other somatic-plate cells, $x^{1.1}$ and $x^{1.2}$, divide again; $x^{1.1}$ synchronously with the division just mentioned (Fig. 38), but $x^{1.2}$ considerably later (Figs. 41–44).* Both these divisions are clearly contrary to the rule of alternating cleavage, for both are in the *same direction as that of their parent cell* x^1 (Fig. 20).

A glance at Figs. 38–40 is sufficient to show that the somatic-plate cells begin early to arrange themselves symmetrically with regard to the middle line of the embryo: X_3 is squarely in the middle line, x^3 nearly so (see future divisions); x^2 on the left side balances $x^{1.1}$ on the right and divides symmetrically with it. $x^{1.2}$, however, does not have a corresponding cell on the other side. It is worthy of note that in many animals this small cell arises in the same way, and has about the same proportional size (Unio,[3] Fig. 41, *Nereis*,[1] Fig. 55, *Clymenella*, Fig. 78, *Chætopterus*, Fig. 30).

I have watched the cleavage of the somatic plate from two principal points of view: that of developmental mechanics, and that of the axial relationships, the shifting of areas, etc.

The bilateral divisions of the somatic plate. — We have seen that up to the 64-cell stage all the divisions took place strictly in accordance with the rule of alternating oblique cleavage, with no regard to bilaterality. But after this stage the cells of the somatic plate divide *without regard to the rule of alternating cleavage, and with marked bilaterality.* Of the two divisions described last, that of x^2 might possibly be considered as a continuation of the alternating cleavage (Fig. 38). The division is oblique and to the right; but since it has its mirrored image in the division of $x^{1.1}$, I believe, to state it paradoxically, that x^2 divides obliquely to the right for the same reason that $x^{1.1}$ *does not* — to conform to bilateral symmetry (Fig. 38, text Fig. IV).

* I have seen this cell in process of karyokinesis many times.

The cell X_3 divides meridionally and bilaterally, and the resulting cells, equal in size, lie one on either side of the

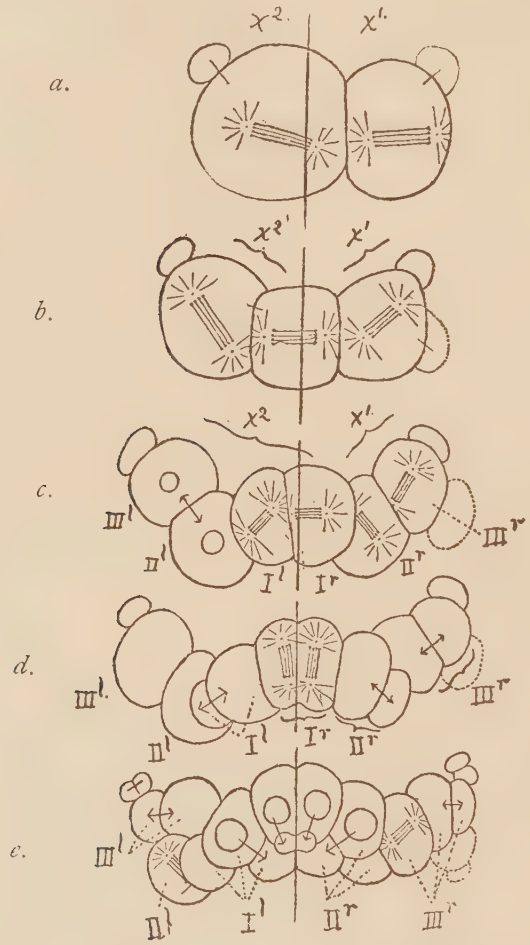

Fig. IV. — Diagram of a group of somatic-plate cells. The perpendicular lines indicate the sagittal plane of the trochophore. Figs. *a* and *b* show the origin of the bilateral group of cells in *c*, where I^l, II^l, III^l on the left correspond in position to I^r, II^r, III^r on the right, although the sagittal plane passes through I^r. After the subsequent divisions the group is still bilateral, but the descendants of the cells I^l, II^l, and III^l no longer correspond in position to those of I^r, II^r, and III^r. The four cells in *e*, whose nuclei are indicated, constitute the paratroch.

median line of the embryo. The later divisions of these cells are strictly bilateral as far as I have followed them (Figs. 39, 40–52, cf. p. 245).

The succeeding bilateral divisions of $x^{2.2}$ and $x^{1.1.2}$ are of special interest, for, although the cells, as a group, are not placed quite bilaterally with respect to the embryo, their next division corresponds in direction (Figs. 41, 42 and text Fig. IV, *a*, *b*). The products of $x^{2.2}$ are subequal, and of about the same size as the adjacent larger product of $x^{1.1.2}$, the latter having divided unequally. This division gives the impression of an effort towards bilateral cleavage in cells not perfectly balanced with respect to the middle line. As a result, the three subequal cells lie in a transverse row, one in the median plane. The small cell on the extreme right apparently has little to do with the balance of symmetry: it has no fellow on the left side (Figs. 41, 42 and text Fig. IV, *b*).

The middle cell of the group divides as an unpaired median cell usually does in bilateral cleavage, *i.e.*, *meridionally and equally;* the cells on either side of it divide bilaterally — the division of either being the mirrored image of that of the other. As a result, we have six cells arranged in a bilaterally symmetrical group, *whose plane of symmetry falls slightly to one side of the middle line of the embryo* (Figs. 43–47, text Fig. IV). This being the case, it is obvious that, if the next division should take place *symmetrically with reference to the middle furrow of the group, the arrangement of the resulting cells would be asymmetrical with reference to the middle line of the embryo.* And conversely, if, after the next division, the cells should be arranged *symmetrically with regard to the middle line of the embryo, they must have divided with total disregard of the symmetry of the group.*

Which course will the cleavage pursue? As compared with the bilateral cleavage in eggs like the squid and ascidians, where the plane of symmetry of a group of cells coincides with that of the embryo, and where the origin of the cells on one side is the same as that of the corresponding cells on the other, the case we have in hand is very complex. — Here we have a bilaterally symmetrical group of cells somewhat asymmetrically placed, and the origin of the cells on one side is different from that of the corresponding cells on the other; they do not even belong to the same cell generation. To my mind this is a

pretty severe test of the influence of bilaterality of the whole organism upon the cleavage in certain parts. The two subsequent cleavages are therefore of special interest, for they give to the question an unequivocal answer. The next division takes place with *total disregard of the symmetry of the group*, and results in a new arrangement of the cells, which is *symmetrical with regard to the middle line of the embryo* (Figs. 46–52 and text Fig. IV, *c*, *d*).

The middle cells I^l and I^r are sister cells, equal in size and symmetrically placed in the group, but they divide very differently (text Fig. IV, *c*): I^r divides like an *unpaired median cell* and its equal products lie one on either side of the median line of the embryo; I^l divides symmetrically with II^r; III^r divides so that its products correspond to the (undivided) cells II^l and III^l. The grouping is thus rearranged, a new symmetry established, and the middle line of the new group coincides with that of the embryo. This is shown to be true, not only by the position of the cells, but by their subsequent divisions: corresponding cells *divide symmetrically* — bilaterally (Figs. 49–55 and Diagram III, *d*, *e*).

The four larger products of the four median cells are the *paratrochal cells*. They never divide again, and constitute the perianal paratroch of the trochophore, which persists as a ring of ciliated cells around the body just in front of the anus, until the larva has developed five or six metameres (Figs. 53–59, 61–64, light brown, text Fig. IV, *c*, with nuclei). The smaller products of these cells lie within the arc formed by the paratrochal cells and give rise to those structures only, which are posterior to the paratroch,—the proctodœum, etc. Since they mark the *posterior end* of the larva, I have called them *terminal cells*.

The following facts in regard to the origin of the paratrochal cells and the enclosed terminal cells, though obvious, are too important to be left unmentioned:

(1) *They all arise from the somatoblast X.* (2) *They lie at the posterior lip of the blastopore or entoderm plate.* (3) *At first the four lie nearly in a straight row, only the two dorsal ones meeting in the middle line.* (4) *Three of the four are*

descended from x^2, *the remaining one* (*right ventral*) *from* x^1: *in other words, the material of three of the cells was separated from that of the fourth, at the first division of the somatoblast* X (d^2, *16-cell stage*), *and, as we have seen, has had a different experience.* (5) *Notwithstanding the differences in their past history, the four cells are symmetrically located and are of the same generation* (*11th generation remote from the ovum*).

To return to the somatic plate at the 64-cell stage—X_2 divides to form x^3 and X_3: X_3, the large cell, divides into two equal cells, lying one on either side the middle line; X_{3r} and X_{3l}, and their products divide bilaterally, and are symmetrically placed, Figs. 43–55; X_3 and X_3 divide into X_4 and X_4, and x^α and x^α; X_4 and X_4 into X_5 and X_5, and x^β and x^β; X_5 and X_5 into X_6 and X_6, and x^γ and x^γ. After this x^3 divides very unequally into $x^{3.1}$ and $x^{3.2}$; then X_6 and X_6 divide into X_7 and X_7, and x^δ and x^δ. At about this time x^β and x^β, x^γ and x^γ, and $x^{3.2}$ all divide bilaterally and equally.

The cleavage of the remaining ectoderm cells in the posterior hemisphere. — We have already described the fate of the secondary trochoblasts, page 240, and showed that, with the exception of three cells not much larger than polar globules, all their descendants enter into the prototroch (cf. the latero-dorsal trunk region in *Nereis*,[1] pp. 419, 427).

The remaining ectodermal cells fall naturally into groups; those descended from $a^{2.2}$, $b^{2.2}$, $c^{2.2}$, and from a^3, b^3, c^3, and d^3 of the 32-cell stage, — we have already described the division of each of these cells in the left oblique direction. There is little to be gained from a detailed description of the further cleavage of $a^{2.2}$, $b^{2.2}$, and $c^{2.2}$, though I followed it in the eggs in order (1) to be sure of the boundaries of the groups, (2) to become acquainted with any landmarks which might develop, (3) to see whether in these late stages there is the same constancy in the direction of division and in the size of the blastomeres in different individuals, that obtains in the earlier stages, and (4) to obtain data for close comparison with other species. (1) In Fig. 52 the boundaries of these groups are indicated. (2) The rows of small cells with deeply staining nuclei, not far from the edge of the somatic plate, are important landmarks

(Fig. 57). (3) In the late stages of cleavage, as in the early ones, all individuals of the species are alike. (4) The upper product of $a^{2.2}$, which forms the larval mesoblast in *Unio*, is in *Amphitrite* minute (Figs. 41–44), but its exact fate is unknown. The lower products of $a^{2.2}$, $b^{2.2}$, $c^{2.2}$ come to lie in about the same position as the "stomatoblasts" in *Nereis*, but I do not know their ultimate destiny. In each of the three groups, even at a late stage, one or two of the cells are of comparatively large size, and the rest very small; the former correspond in position to Wilson's "stomatoblasts" (Wilson,[1] p. 414).

In reference to the products of a^3, b^3, c^3, a^3, it need only be said that they behave in the same manner in all individuals, through several generations, and form important landmarks (cf. Figs. 41–62).

Entoderm. — At the 64-cell stage the entoderm consists of seven cells, which occupy a large part of the surface on the posterior hemisphere. They are A_4, B_4, C_4, D_4, and a^4, b, c^4, (d^4 being the mesoderm cell). The four cells first mentioned all divide again, and except for D_4, which divides obliquely to the right, and in advance of the others, they all cleave practically in the same direction as did the parent cells. The relative size and position of the resulting cells is remarkably constant in every egg. In consequence of these divisions the *entoderm plate* assumes the form of a cross, consisting of eleven cells arranged in the following manner:

$$\begin{array}{ccccccccc} & & & & d^4 = M - M = d^4 & & & & \\ & & & & d^5 & & & & \\ a^4 & a^5 & A & \Big\rangle & \dfrac{D_5}{B_5} & \Big\langle & C_5 & c^5 & c^4 \\ & & & & b^5 & & & & \\ & & & & b^4 & & & & \end{array}$$

The surface area of the cross gradually diminishes as the cells elongate at right angles to the surface.

None of these cells divide again until after they have migrated into the segmentation-cavity, where they give rise to the entoderm of the larva (Figs. 43–56, text Fig. VI).

Mesoderm. — The mesoderm cell $d^4 = M$, of the 64-cell stage, divides very early into two equal cells bilaterally placed upon

the surface (Fig. 38). These two cells, the anlagen of the paired mesoderm bands, immediately begin to elongate, and soon become cylindrical in shape. The nucleus of each migrates inward, and when it nears the middle of the cell, forms a karyokinetic spindle, and a very minute cell, *m*, is "budded

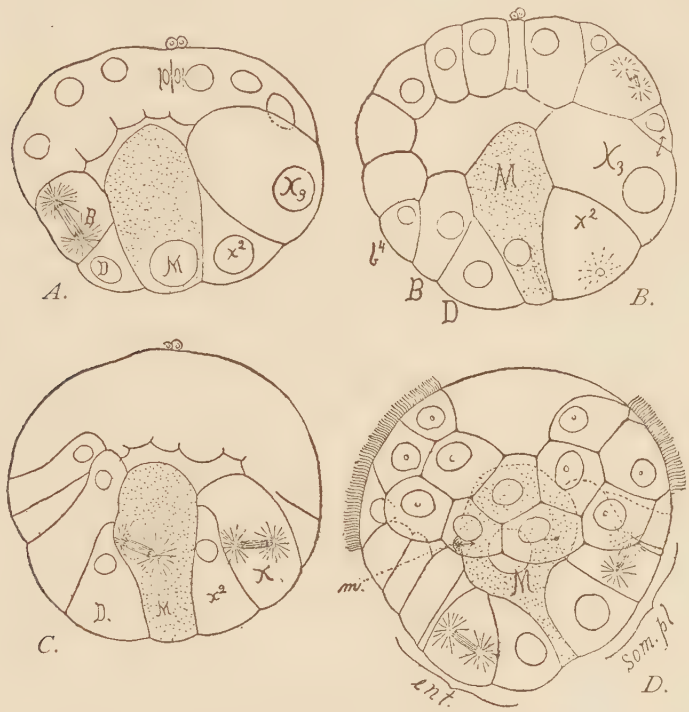

FIG. V. — Optical sagittal sections from camera sketches, showing invagination of the mesoderm. *M*, mesoderm; *m*, the minute cell, product of the division represented in *C*; *D*, *B*, b^4, ent., entoderm; X_3 X^2, som. pl., somatic plate. In *D* the left half of prototroch is represented; those cells in which the nucleoli are drawn belong to the *primary* prototroch, the rest are the secondary prototrochal cells.

off" ventrally. These two small cells seem to correspond to those arising by the first division of the *M* and *M* in *Nereis*, *Unio*, and other forms. The peculiar thing about the division in *Amphitrite* is, that instead of taking place on the surface, it occurs after the mesoderm cells have begun their inward migration, but before they are wholly inside the segmentation cavity, and the spindles lie in the *short diameter of the cells*. Furthermore, the mesoderm cells, just at the time when the division

occurs, are squeezed in between the large somatic-plate cells on the dorsal side, and the large entoderm cells on the ventral side. In fact, no other cells seem to be under greater pressure at any period of development, and yet the axes of the spindles lie in the direction of greatest pressure (Figs. 41, 44, text Fig. V, *c*).

The two larger cells can be seen at the surface for a time after this division, but soon disappear within the segmentation cavity (Figs. 41–44, 46, 50). Once inside, they quickly assume a spherical form, having the minute cells *m* and *m* still attached to them (Fig. 50), and meet in the median plane. They behave like teloblasts, and give rise to the two diverging rows of mesoderm cells which, considerably later, break up into bands several cells wide. The cells *m* and *m* can be plainly distinguished until the mesoderm cell-rows contain five or six cells each (Fig. 52).

Summary. — With the attainment of the 64-cell stage the rhythmical, alternating, oblique cleavage suddenly stops. Some cells never divide again, some divide bilaterally, some continue to divide in the alternating oblique direction.

The formation of the apical rosette and cross is remarkably similar to that in *Nereis limbata.*[1] The middle cells of the dorsal arms of the cross, which become the "head kidneys" in *Nereis*, become large mucous glands in *Amphitrite*, while the corresponding cells of the ventral arms probably have a similar destiny. The sixteen cells which are the descendants of the *primary trochoblasts* $a^{1.1.}$, $b^{1.1.}$, $c^{1.1.}$, $d^{1.1.}$, of the 16-cell stage (Pl. X, Fig. 11), *all* acquire cilia and constitute the *primary* prototroch (Fig. 18). They are arranged in four groups, which are separated by non-ciliated areas. A little later the cells filling three of these interspaces, the two lateral and the ventral ones, become ciliated, so that a band of cilia surrounds the larva, except in the mid-dorsal line. This completed prototroch consists of twenty-five cells, — sixteen from the upper hemisphere, and nine from the lower. Later in the development the dorsal interruption is obliterated by the concrescence of the prototroch cells from either side, without the addition of other cells. While the interruption still persists, the

undifferentiated ectoderm cells of the upper and lower hemispheres are in free communication at this place, and a few migrate from a position above the prototroch to one below it.

From the 64-cell stage nearly all the cells of the large dorsal quadrant behave very differently from those in the other three quadrants. This is especially noticeable in the descendants of the somatic-plate cells (d^2) and the mesoderm cell (d^4). They do not contribute to the prototroch as do those of the other three quadrants, and they manifest a marked tendency to cleave and arrange themselves bilaterally with respect to the middle line of the embryo.

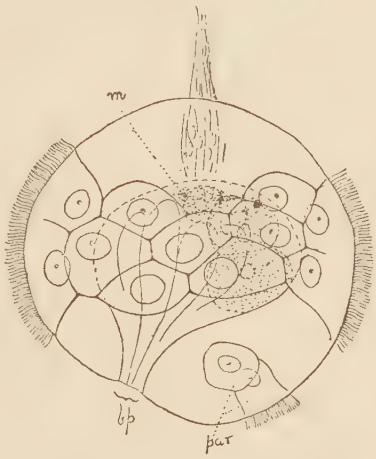

FIG. VI.—Optical sagittal section of trochophore at the time of the closure of the blastopore, with surface view of prototroch and paratroch (par.). Outline of segmentation cavity indicated by dotted line; mesoderm stippled; *m*, minute product of first division of paired mesoderm cells; *bp*, blastopore; the prototrochal cells with nucleoli are from primary, the others from secondary, trochoblasts. From camera sketch.

The paratroch is differentiated at a comparatively early stage and is composed of four cells, descendants of a^2 (Pl. X, Fig. 14), which at first lie in a very slightly curved arc at the posterior or dorsal lip of the blastopore. A few small cells, likewise descendants of d (X), also lie within this arc, and, together with the paratrochal cells, mark with absolute certainty the posterior end of the larva (Pl. XIV).

The *mesoblast cell*, d^4, divides once at the surface, and the two resulting cells sink into the segmentation cavity, where they function as teloblasts and give rise to a pair of mesoblast bands. The first division of each, however, occurs during the inward migration, and the spindles lie in the short diameter of the elongate cells, and apparently in the direction of the greatest pressure. The fact that in other forms similar divisions occur at the surface renders this cleavage especially significant (text Figs. V, VI, and IX).

The division of the four cells lying at the vegetative pole (A_4, B_4, C_4, D_4) forms a definitive, cross-shaped, entoderm plate of eleven cells.

The trochophore consists of about two hundred cells when the paratroch is differentiated, while the mesoblast bands, at this time, are made up of four cells each.

III. Formation and Elongation of the Trunk.

a. *Shifting of areas on the lower hemisphere.*— The present chapter will consider cleavage only as a means of orientation in following the movements of embryonic areas, during the metamorphosis of the spherical one-layered trochophore into the elongated three-layered larva.

The prototroch naturally divides the trochophore into an anterior umbrellar and a posterior subumbrellar region. Upon the latter the more important shifting of areas occurs, involving the somatic plate, colored blue ; the mesoderm, red ; the entoderm, stippled; and the rest of the ectoderm, untinted. At a stage when the mesoderm cells are still at the surface (Figs. 38, 40–42), they, together with the entoderm cells, occupy a relatively large area on the subumbrella, even larger than that of the somatic-plate cells.

The mesoderm sinks into the segmentation cavity, and the somatic plate, by spreading out, comes to lie next to the posterior edge of the entoderm plate. In the median line the point of meeting of the two plates is about 90° from the prototroch (text Fig. VII). The eleven entoderm cells begin to invaginate in exactly the same manner, and consequently the surface area of the entoderm plate gradually diminishes until it finally disappears. Since the cells all invaginate at a uniform rate, the pattern of the plate on the surface remains nearly the same in shape, though constantly diminishing in size. The blastopore closes from all directions at once, and the stomodæum is formed where the entoderm cells were last seen on the surface, *i.e.*, about 30° behind the prototroch in the mid-ventral line (later, however, when the œsophagus is formed, the mouth

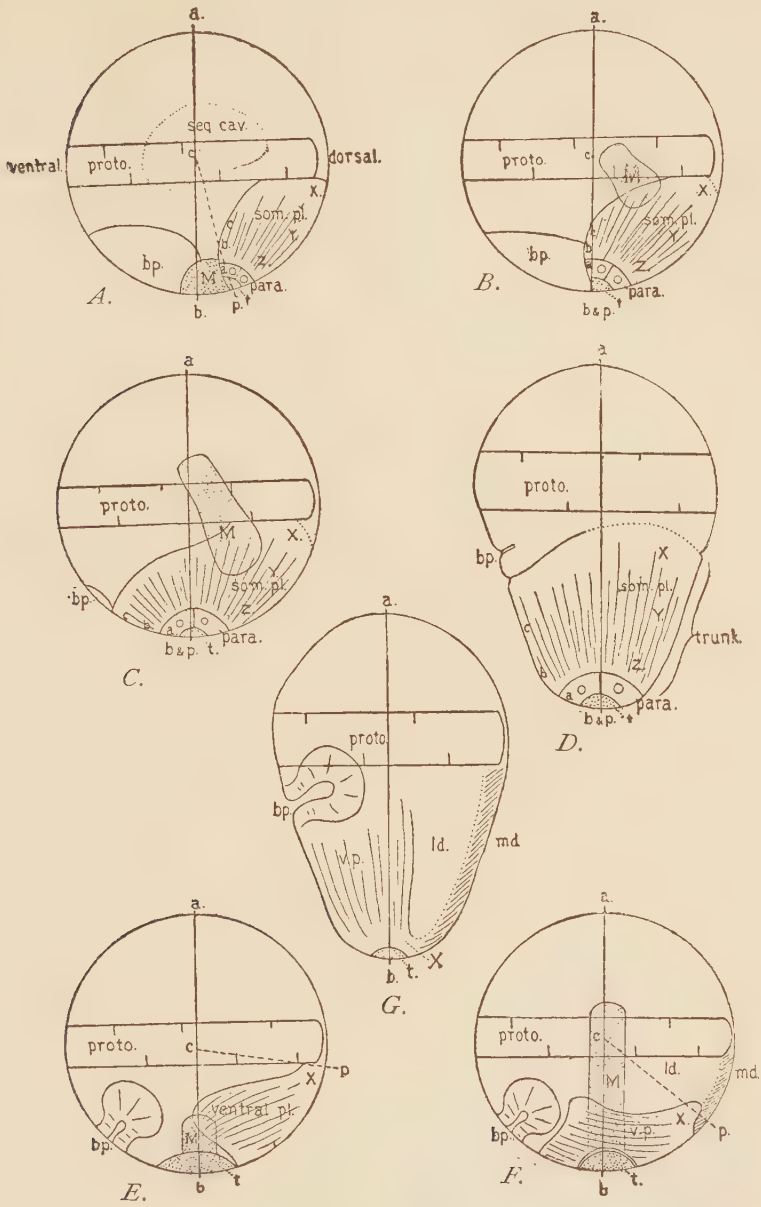

FIG. VII. — Diagrams to express the essential difference in the axial relations and the manner of formation of the trunk between *Nereis* (Wilson[1]), and *Amphitrite* (this paper). *A*, *B*, *C*, and *D*, *Amphitrite*; *E*, *F*, and *G*, *Nereis*. Line *acb*, egg axis; line *acp*, anterior posterior axis; *bp.*, blastopore; *M*, mesoderm; *m.d.*, mid-dorsal region; *l.d.*, latero-dorsal region; *para.*, paratroch; *proto.*, prototroch; *som.pl.*, somatic plate; *t.*, terminal region; *v.p.*, ventral plate; *X*, that part of somatic plate originally nearest the prototroch.

is nearer the prototroch). Thus, the cells which at first occupied a large part of the area of the subumbrella have left the surface entirely and sunk into the cavity. But since the general contour of the egg is but little altered, it is obvious that, meanwhile, other cells must have come to occupy this area. They are those of the somatic plate, and the process in general is simply this: the mesoderm and entoderm constantly sink in and so diminish in surface area; on the other hand, the somatic plate becomes thinner, and extends its surface area until it occupies, not only nearly all its original portion of the subumbrella, but also that of the mesoderm and entoderm.

The manner in which the somatic plate extends into the new area is of especial interest. Upon the invagination of the mesoderm, the posterior border of the somatic plate moves slightly backwards, and meets the entoderm plate at the center of the subumbrella, — the point *b*, text Fig. VII, *A*, *B*. This is the only backward movement which occurs on the border of the somatic plate *in the middle line*. The material at *b* always remains 90° from the prototroch, and finally becomes the posterior end of the metameric larva. The border of the plate on either side continues to move round this pivotal point: its outline is at first convex, but soon becomes nearly straight, then V-shaped, and finally the edges on either side meet and concresce in the mid-ventral region of the embryo (text Figs. VII and VIII).

In this movement the material which shifts its position does not change its *latitude*, *i.e.*, the material of the plate which was at first nearest the prototroch remains always nearest; that nearest the point *p*, or farthest from the prototroch, remains always in this relation, and forms the posterior end of the embryo.

So it comes about that the somatic-plate cells form a cap over the whole posterior portion of the subumbrella. The only other ectoderm cells are in the region of the stomodæum and the few small cells, which, as we have seen, migrated from the upper hemisphere through the interruption in the prototroch. These occupy at most but a limited area just posterior to the prototroch.

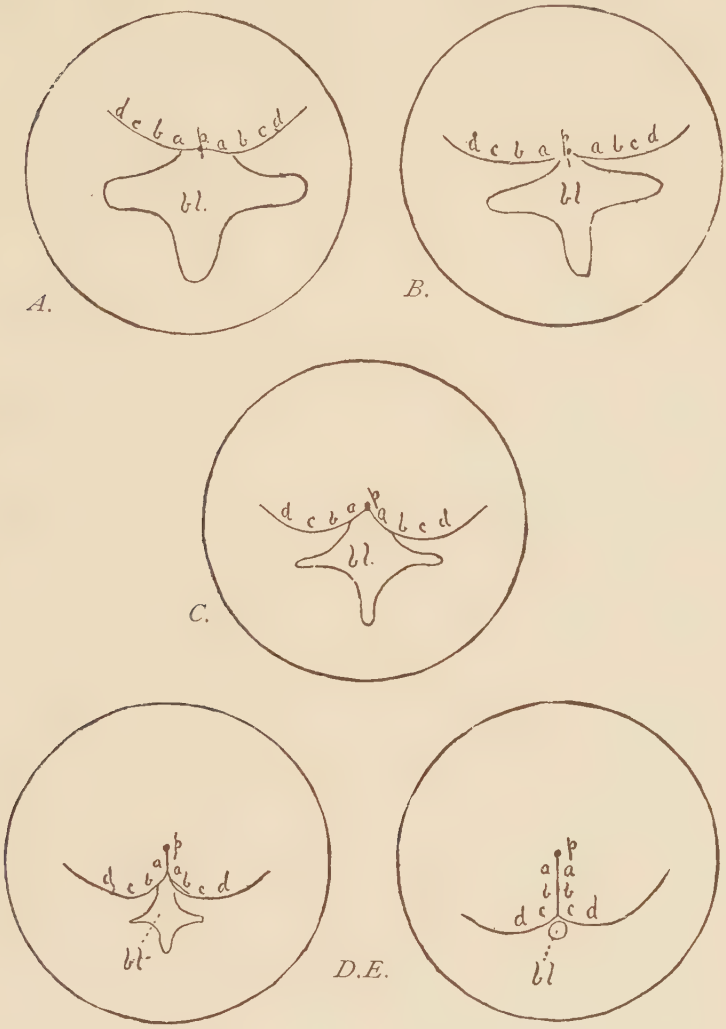

FIG. VIII. — Diagrams of subumbrella showing the lateral extension of the borders of the somatic plate on either side and their final concrescence in the mid-ventral line. The point *p* is the posterior end; *bl.*, the blastopore; the lines *a*, *b*, *c*, *d*, the ventral border of the somatic plate. The material at *p* does not move, but that at *a*, *b*, *c*, *d*, on either side, moves round as the blastopore closes, and meets that of the other side in the mid-ventral line. As a result the point *p* is entirely surrounded by cells of the somatic plate.

The figures on Plates III, IV, V show the various stages in the lateral extension of the somatic plate.

Formation of metameres. — By the time the blastopore has closed, the paratroch has become conspicuous, and has nearly attained its ring-like form (Figs. 58, 62). (This is not perfected, however, until after the invagination of the proctodœal cells — about Fig. 64.)

The subumbrella now begins to elongate rapidly owing to the further cleavage of the cells just in front of the paratroch — the budding zone.

The constant parallelism of the prototroch and paratroch indicates clearly the amount of elongation of the trunk region, and shows that it grows on all sides, dorsal, ventral, and lateral, with equal rapidity. The consequent changes of contour are illustrated in the text Figs. X–XVIII. The ectoderm of the trunk becomes thinner and thinner as the latter elongates. The first indication of metamerism is a groove which appears a little behind the prototroch (Fig. 64), and separates the head segment from that of the trunk. I believe that all the ectoderm posterior to this groove arises from the somatic plate, or, in other words, from the descendants of cell d^2 of the 16-cell stage; it is possible that a part of the region in front of the groove is also occupied by somatic-plate cells.

After further elongation, the trunk divides into two segments. The posterior elongates and divides, thus giving rise to three trunk segments. The subsequent elongation of the body is accompanied by the repeated division of the ultimate metamere. In text Fig. XVII, four setigerous segments are outlined, though the prototroch and paratroch still persist at opposite ends of the larva.

No ectodermal teloblasts are distinguishable at any time during the formation and elongation of the trunk.

Although I have not made a complete study of the structures of the late trochophore and the development of the larval organs, I will record the following observations.

Alimentary tract. — The cells of the entoderm plate sink into the segmentation cavity in the manner described above, without producing any marked depression on the surface of the egg (Figs. 61, 62, text Fig. VI). Here they commence to divide and form a solid mass of cells, which, with the mesoderm, completely fills the segmentation cavity.

The ectoderm cells in the vicinity of the blastopore form an œsophagus which acquires a lumen, while the cells of the entoderm are still in a solid mass (Figs. 63, 64). Considerably later the proctodœum is formed from cells within the paratrochal ring which originally lay at the posterior lip of the blastopore, and belonged to the somatic plate (p. 244). About this time the mass of entoderm cells acquires a lumen. The gut is differentiated into a stomach and intestine, and in the walls of the former is found nearly all the yolk.

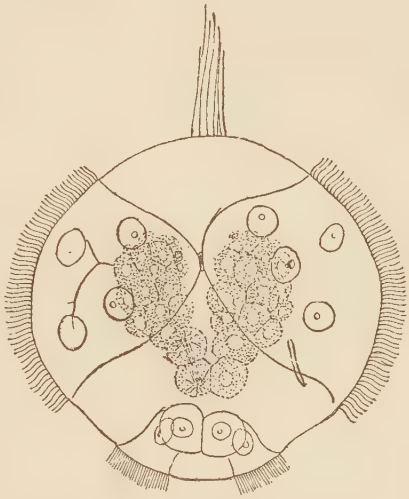

Fig. IX. — Trochophore from the dorsal side, showing prototroch, paratroch, apical tuft, and mesoderm bands within (stippled). From camera sketch.

Fig. XVIII, p. 261, representing a larva eleven days old, shows that the œsophagus, stomach, and intestine are already highly differentiated. The œsophagus is ciliated throughout, and there are numerous strong cilia in the stomach near the narrow cardiac opening.

Mesoderm. — When we last referred to the mesoderm there were four cells in each band (Fig. 61). For some time the number increases by simple division of the teloblasts, and then the single rows are broken by cleavage of the component cells. Even after this, however, one can distinguish the teloblasts lying close together and as near as possible to the posterior end of the embryo (text Fig. IX). In the much elongated larva one can make out in section the mass of undifferentiated mesoderm at the posterior end, and anteriorly the well-defined mesoderm layers lining the gut and body-wall. There is no persistent primary body-cavity.

Mucous glands. — After it is no longer possible to follow exactly the arrangement of the other cells of the upper hemisphere, the dorsal mucous glands *gl.r.* and *gl.l.* can easily be traced, since their nuclei are particularly large and clear and

have peculiar nucleoli (Fig. 59). I have paid less attention to the glands on the ventral side. At a stage like that of Fig. 64 and text Fig. X, the gland cells present a new appearance. The outer end of each cell (the end of each duct), becomes filled with a substance which stains very deeply with methyl-green, hæmatoxylin, etc., and a few hours later this substance fills the duct and the upper part of the body of each of the gland cells (text Figs. XI–XVIII). Meanwhile two other pairs of gland cells appear in the head segment just behind the prototroch. They are bilaterally placed; one pair close together on the ventral side just back of the mouth, the other pair also close together on the dorsal side.

When the larvæ from twenty-four to thirty-five hours old are killed in Perenyi's fluid and stained with Biondi Ehrlich, the numerous glands are brought out in brilliant contrast to the other tissues, for the latter are light red, while the glands are an intense green. The body of each gland becomes greatly distended. In larvæ five days old mucous glands appear also in the paratrochal region. All the glands, at least in the head region, are unicellular and ectodermal.

Nervous system. — The two eye spots which are formed at the beginning of the second day persist until at least the eleventh day, — long after the disappearance of the ciliated organs. They are simple structures, consisting of an outer brown pigment, a clear lens, and apparently an optic nerve.

The cluster of columnar cells immediately under the apical tuft represents, I presume, an apical sense organ (Fig. 64). That the brain arises from the "first group of micromeres," *i.e.*, from the four upper cells of the 8-cell stage, — the "encephaloblasts" of von Wistinghausen,[5] — there can be no doubt; but so do many other organs, for example, the mucous glands, and the greater part of the prototroch. In comparatively early stages the fibrous portion of the brain can be made out directly under the apical pole. I do not know from what cells the brain arises, and, while it may come from the cross cells, as

[5] Wistinghausen, C. v.: Untersuchungen über die Entwicklung von Nereis Dummerillii. *Mitth. a. d. Zool. Stat. zu Neapel.*, Bd. 10, 1891.

Wilson holds for *Nereis*,[1] I see no conclusive evidence, for or against this supposition.

The ventral cord is differentiated as usual from the ectoderm of the ventral plate. Ganglia, connectives, etc., are formed while the larva is still in possession of prototroch and paratroch, and the first ventral ganglion lies in the first setigerous segment.

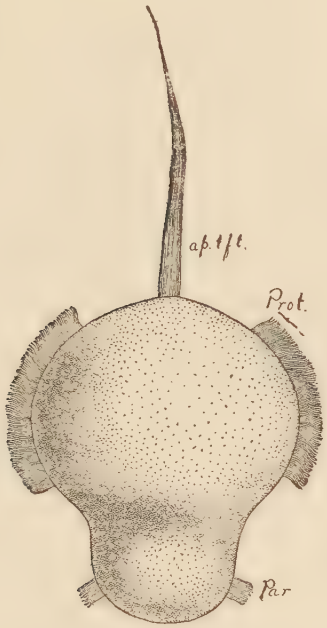

FIG. X. — Trochophore of *Amphitrite*, about 20 hours; *ap. tft.*, apical tuft; *prot.*, prototroch; *par.*, paratroch.

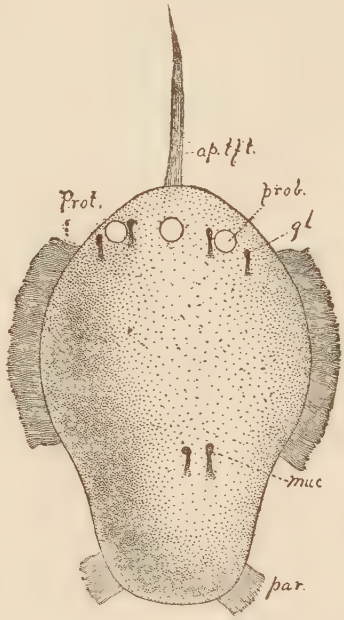

FIG. XI. — *Amphitrite*, 24 hours, ventral aspect; *ap.tft.*, apical tuft; *prob.*, problematic bodies; *gl.*, duct of umbrellar mucous gland; *muc.*, subumbrellar mucous gland; *par.*, paratroch; *prot.*, prototroch.

Problematic bodies. — At about the time the anterior mucous glands begin to react to the methyl-green, there appear in the ectoderm near the openings of the glands five spherical bodies, disposed symmetrically, one on the mid-ventral line, two on the ventral, and two on the dorsal side. They increase rapidly in size up to a certain point, and remain as long as the mucous glands and the prototroch. They appear to be spherical vesicles filled with a fluid, which does not react to methyl-green nor Delafield's hæmatoxylin like the substance of the mucous

glands, but does stain with Zocher's alum-cochineal. I do not understand their structure and function; — possibly they are homologous with the frontal bodies of *Nereis* (Wilson) (text Figs. XII–XVIII).

Cilia. — Cilia are seen first on the sixteen primary cells, and then on all the twenty-five cells of the completed prototroch. Next the paratroch becomes ciliated, and, at about the same time, a tuft appears at the apical pole. The cilia of the prototroch and paratroch at first are very numerous and fine, and, in each case, are distributed in an even zone encircling the

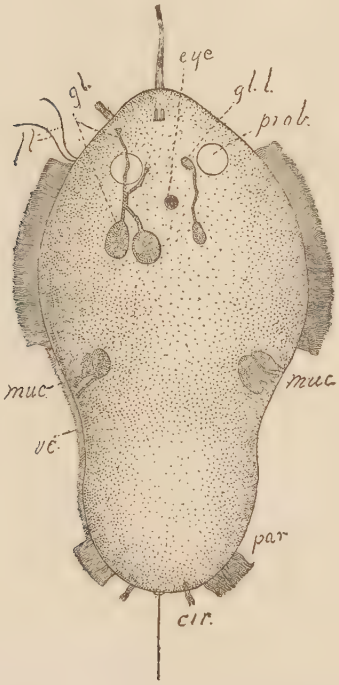

Fig. XII. — *Amphitrite*, 28 hours, left side; *gl.l.*, left dorsal umbrellar mucous gland (opening); *gl.*, ventral umbrellar mucous gland; *fl.*, flagellum; *muc.*, subumbrellar mucous gland; *v.c.*, ventral band of cilia; *cir.*, anal cirrus; *prob.*, problematic bodies.

Fig. XIII. — *Amphitrite*, 36 hours, ventral; *prob.*, problematic bodies; *gl.*, ventral umbrellar mucous gland; *op.*, its external opening; *muc.*, subumbrellar mucous gland; *par.*, paratroch; *cir.*, anal cirrus.

trochophore. Later they become much larger, strong, and so tough that they are often fairly well preserved with so harsh a reagent as Perenyi's fluid. The apical tuft which is very large in

young trochophores, atrophies when the body begins to elongate, and a few clumps of short cilia appear upon the umbrellar surface.

Two or three large slowly moving flagella are found in front

FIG. XIV. — *Amphitrite*, 44 hours, dorsal; *gl.l.*, duct of left dorsal umbrellar mucous gland; *gl.r.*, right dorsal umbrellar mucous gland; *prob.*, problematic bodies, *gl.*, ventral umbrellar mucous gland; *muc.*, ventral subumbrellar mucous gland; *st.*, seta; *par.*, paratroch; *cir.*, anal cirrus; *I*, *II*, *III*, 1st, 2d, and 3d trunk segments.

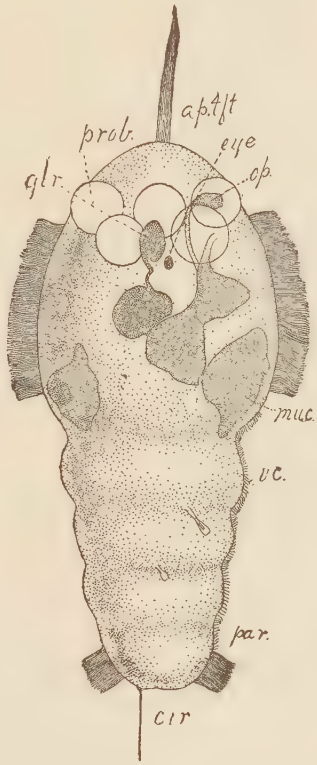

FIG. XV. — *Amphitrite*, 44 hours, right side; *ap.tft.*, apical tuft; *gl.*, and *op.gl.*, ventral umbrellar mucous gland and its external opening; *gl.r.*, right dorsal umbrellar mucous gland; *prob.*, problematic bodies; *muc.*, subumbrellar mucous gland; *v.c.*, ventral band of cilia; *par.*, paratroch; *cir.*, anal cirrus.

of the prototroch in the mid-ventral line. A ventral band of short cilia connects the prototroch and paratroch, and a few ciliary tufts and a large cirrus occur in the region behind the paratroch (text Figs. X–XVIII).

Setæ, parapodia, etc. — When the larva has elongated and has begun to divide up into metameres, it develops in each

segment a pair of oar-shaped setæ, and soon after one or more pairs which are needle-shaped, while the body-wall is evaginated to form parapodia, in which seta-muscles, etc., are developed. Shortly after the dorsal setæ are developed, there appear in all the trunk segments, except the first, some little hooks, the setæ of the ventral parapodia (text Figs. XII–XVIII).

Fig. XVI. — *Amphitrite*, 44 hours, ventral aspect; *prob.*, problematic bodies; *gl.* mucous gland; *muc.*, subumbrellar mucous gland; *st.*, seta; *par.*, paratroch; *cir.*, cirrus.

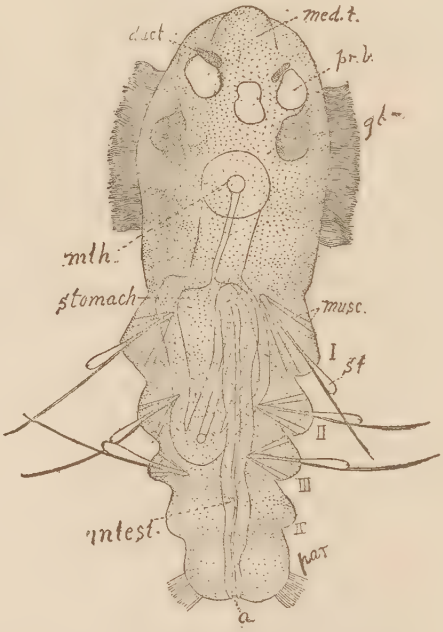

Fig. XVII. — *Amphitrite*, ventral aspect, 60 hours; *med.t.*, median tentacle; *pr.b.*, problematic body; *gl.*, mucous gland; *mth.*, mouth; *musc.*, setæ-muscles; *st.*, setæ; *par.*, paratroch; *a.*, anus; *I*, *II*, *III*, *IV*, trunk segments.

IV. Metamorphosis from Free-swimming to Creeping Larvæ.

When the larvæ have developed about five trunk segments, they no longer swim about freely, but sink to the bottom and become adapted to a less active mode of life, and if delicate pieces of fresh ulva are put into the aquarium, they thrive much better.

The prototroch and paratroch, before their actual disappearance, undergo a marked degeneration. The cells shrink and become filled with yellow granules, and within a comparatively short time all the ciliated tracts on the surface and all the mucous glands disappear. These phenomena, together with the fact that the glands occur in the region of the prototroch and paratroch, indicate that they are correlated physiologically with the cilia. The problematic bodies also partially collapse and then disappear, and a *median tentacle* is formed by an evagination of the body-cavity just ventral to the apical pole. Since this appears before the ducts of the mucous glands and the problematic bodies have entirely degenerated, it is a valuable means of orientation in later stages (text Figs. XVII, XVIII).

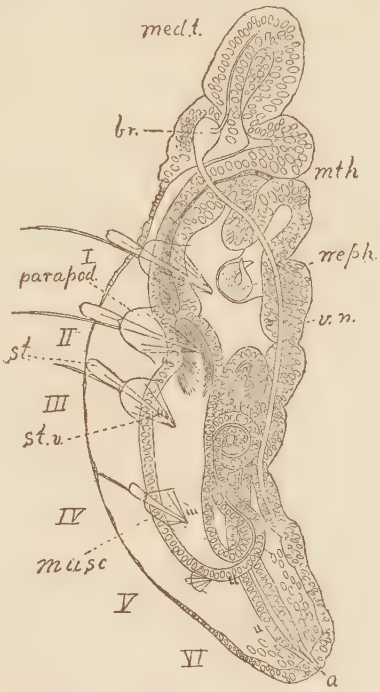

FIG. XVIII. — *Amphitrite*, 11 days, optical section: *med.t.*, median tentacle; *br.*, brain; *mth.*, mouth; *neph.*, nephridium; *v.n.*, ventral nerve cord; *parapod.*, parapodia; *st.*, seta (dorsal); *st.v.*, setæ (ventral hooked); *musc.*, muscles of setæ; *I, II, III*, etc., trunk segments.

Text Fig. XVIII shows the large mouth, the position of brain, cord, eyes, nephridia, etc.

B. CLYMENELLA TORQUATA VERRILL.

Habits and cleavage. — The breeding habits of *Clymenella torquata* in the vicinity of Woods Holl are very different from those of *Amphitrite*. While the latter may be found breeding at any time during the summer, but never in great abundance, the breeding season of *Clymenella* lasts but two or three days, varying in different years from the last of April to the middle of May, and almost every normal individual discharges its sexual products at this time. The worms which are found

nearly everywhere in light sand or gravel just below low-water mark, live in slender sand-tubes.

Under the most favorable circumstances I have sought in vain for the eggs deposited under natural conditions, and believe they are discharged free into the water, usually at night, and scattered by the tides.

Since one is liable to be prevented by the high winds prevalent in May from getting the worms at just the right time, the surest way to secure the eggs is to collect a large number of worms just a few days before the breeding season. The males and females can be distinguished, since the eggs show plainly through the body-wall. If the females are kept in an aquarium without sand, the eggs will never be laid, though the worms may live for weeks; nor can the eggs be fertilized, under these circumstances, if cut out of the body-cavity. If, however, an abundance of sand be furnished, they build new sand-tubes, and deposit their eggs on the surface of the sand at the mouth of the tube.*

E. B. Wilson describes the *Clymenella*, which occurs in great numbers at Beaufort, N. C., as laying its eggs in jelly masses the size of pigeon's eggs. These masses are attached to one of the two openings of a Y-shaped tube, and are found all summer in great abundance. In the Woods Holl form many of the Y-shaped tubes are found in the breeding season, but there are no jelly masses. The wide divergence in the breeding habits of the northern and southern forms is remarkable, though it is possible that the worm which Wilson describes is *Axiothea mucosa*, and is generically different from the northern *Clymenella torquata* (cf. Andrews [6]).

The unfertilized egg is practically spherical, about 150μ in diameter, and perfectly opaque, being filled with a large amount of yellow yolk. They may remain unfertilized in the sea-water for several hours at least and still be capable of developing normally.

* If finely broken glass be substituted for sand, they will use this in the construction of their tubes.

[6] Andrews, E. A.: Report upon the Annelida Polychæta of Beaufort, N. C. *Proc. U. S. Nat. Mus.*, vol. XIV, p. 277.

The egg is oriented in precisely the same way as is *Amphitrite*. Up to the time when the sperm enters the egg, the first maturation spindle is in the equatorial-plate stage, and the polar globules remain to mark the apical (animal) pole. The first furrow depresses the surface first at the animal pole, as in *Nereis*.

A detailed account of the later cleavage processes in *Clymenella* would be little else than a repetition of the above description of *Amphitrite*. *Every division up to the 64-cell stage takes place in the same direction in both species.* There is, moreover, a most striking resemblance in the relative size of the cells, though the differences demand attention: the cell D_2 is larger in proportion than in *Amphitrite;* the four upper cells of the 8-cell stage, and therefore the whole anterior hemisphere of *Clymenella* is relatively smaller — among themselves, however, the cells in this hemisphere have about the same relative size as in *Amphitrite;* the mesoderm cell M is comparatively large; the sixteen descendants of the trochoblasts (16-cell stage) have the same arrangement, and *all* become prototrochal cells in the same manner; the apical rosette and cross arise in the same way; the seven cells, which at the 64-cell stage constitute the entoderm plate, are in the same position; the relative size of $a^{2.2}$, $b^{2.2}$, $c^{2.2}$, and $x^{1.2}$ ($d^{2.1.2}$) is the same. x^2 ($= d^{2.1.2}$), however, is somewhat smaller, and this difference may be of considerable theoretical importance in connection with the formation of the paratroch, since three-fourths of the paratroch in *Amphitrite* is formed from this cell (Pl. XV).

Remarkable as are the similarities between the early cleavage stages in these two worms representing rather distantly related families (Maldanidæ and Terebellidæ), some of the subsequent phenomena show even more striking resemblances. The apical rosette cells divide in the same manner (Figs. 71, 72). Each of the "secondary trochoblasts," $a^{2.1}$, $b^{2.1}$, and $c^{2.1}$ divides into a group of four cells: three larger and subequal, and the fourth minute. These groups correspond in the three quadrants. *The three larger cells in each group become ciliated and complete the prototroch, while the minute one does not enter it. Thus, the whole prototroch in Clymenella arises in precisely*

the same way and from the very same cells as in Amphitrite (Figs. 80, 81, 83, 85, 86, text Fig. VI).

The lowest cells A_4, B_4, C_4, D_4 (64-cell stage) divide once more on the surface as in *Amphitrite*, resulting in a definitive entoderm plate of eleven cells. The position of these, however, is slightly different: a^4 and a^5 border upon A_5 in *Clymenella*, while only a^5 does so in *Amphitrite*. The same arrangement obtains in the quadrants *b* and *c*. A_5, B_5, and C_5 each develops a large "vacuole," which can be followed for a long time after the invagination.

Figs. 78–88 show that the divisions of the somatic-plate cells, and those of the other ectoderm cells, $a^{2.2}$, $b^{2.2}$, $c^{2.2}$, and a^3, b^3, c^3, as far as they have been followed, agree closely in the two forms. The paired mesoderm cells *M* and *M* divide *about equally upon the surface*, while in *Amphitrite* this division is very unequal (p. 248).*

The trochophores of the two annelids have a general resemblance, though *Clymenella* is less active.

Summary. — Though the egg of *Clymenella* is much larger than that of *Amphitrite*, and though the worms belong to entirely different families, the similarity in the cleavage is very remarkable.

Up to the 64-cell stage all the cleavages correspond in direction, and within certain limits the relative size of the cells is the same. In the later stages, there is still a wonderful resemblance even in details; the formation of the rosette, cross, and prototroch, and the division of the somatic plate up to eleven cells at least is identical. The entoderm (?) and the mesoderm (?) is derived from the same cells in both forms.

The axial relationships as far as followed are the same in both annelids.

Clymenella differs from *Amphitrite* in the absolute size of the egg, in the relative size of the anterior and posterior hemispheres, the larger size of d^4 (mesoderm cell), the earlier division of *M* and *M on the surface*, the relative size of the products of this division, and the smaller size of X^2.

* Of course in calling these cells *entoderm* and *mesoderm*, I am presuming that their destiny as well as their origin is like that of other forms.

C. LEPIDONOTUS SP.

Habits and cleavage. — The breeding season of *Lepidonotus* at Woods Holl extends from the last of April nearly to the first of June.* The adult worms are commonly found under stones and mussel-beds, and are easily captured, for, when disturbed, they do not attempt to escape, but roll up like porcupines, and depend on their tough dorsal scales for protection. There is no difficulty in separating the males and females, for the former are whitish while the latter are dark on the ventral surface.

It seems that the females may carry the fully ripe eggs for a long time before laying them, for nearly all those captured, even *early* in the breeding season, can be induced to deposit their eggs the day they are collected.

In captivity the worms will rarely lay in the daytime. At night, more frequently from 8 to 10 o'clock, they can usually be persuaded to discharge their eggs, if they are suddenly plunged into colder water, and held up close to the lamp.

The animals remain at the bottom of the dish, and, except for a slight tremor, do not move, while the eggs or sperm stream in delicate threads from the eighteen pairs of nephridial pores. As in the two species described above, the eggs may be kept in sea-water several hours before fecundation. I have fertilized eggs at 8 o'clock in the morning which were laid some time during the night by isolated females, and once purposely kept eggs in sea-water from 5 until 8.30 P.M. before fertilizing them. They developed perfectly well in both cases.

When first laid, the eggs are quite opaque, and very irregular in form. They soon become approximately spherical and about 65μ in diameter. The rapidity of development depends directly upon the temperature of the water (see further on p. 269): at 8° C. the eggs reach the 2-cell stage in about 2½ hours after fertilization; the first cleavage furrow is completed in five minutes and sinks in all round the eggs at the same time, and not first at the animal pole, as in many eggs with abundant yolk like *Nereis* and *Clymenella*. Fig. 89 illustrates the point.

* They may breed earlier.

It represents four superimposed profile views of the egg during the first cleavage, drawn at intervals of about one minute.

The division of the first two blastomeres is equal, simultaneous, and slightly oblique, so we have a 4-cell stage in which the quadrants cannot be distinguished from one another. The cross-furrows at the vegetative and animal poles are about equal in length, and at right angles to each other, so that, as in *Amphitrite*, two of the diagonally opposite cells are higher than the other two. These four cells — the anlagen of the four quadrants — divide simultaneously into eight cells by a right-oblique cleavage (Figs. 90, 91), those of the anterior hemisphere being slightly smaller than the other four. The next division is synchronous and left oblique, and the resulting sixteen cells are nearly equal in size. They soon divide obliquely to the right, but not all exactly at the same time, though the corresponding cells of the four quadrants, or, as Kofoid tersely expresses it, "the cells of the same quartette," divide simultaneously: the upper quartette $a_{1.1.}$, $b_{1.1.}$, $c_{1.1.}$, $d_{1.1.}$, and that at the vegetative pole, A_2, B_2, C_2, D_2, divide first; next the trochoblasts $a^{1.1.}$, $b^{1.1.}$, $c^{1.1.}$, $d^{1.1.}$; and shortly after $a^{2.}$, $b^{2.}$, $c^{2.}$, $d^{2.}$. In Fig. 92 the first two quartettes have already divided, and the two last are dividing. When these divisions are completed, the egg is in a typical 32-cell stage, and the cells are of about the same size, except the four larger at each pole.

While the egg remains in the 32-cell stage the polar globules, which are still attached to the egg at the animal pole, usually penetrate into the cells $a_{1.2}$, $b_{1.2}$, $c_{1.2}$, or $d_{1.2}$. They may enter the same, adjacent, or even opposite cells, and rarely one works its way between the cells into the segmentation cavity. Curiously enough this phenomenon always takes place during the 32-cell stage.

The thirty-two cells all divide obliquely to the left, and the corresponding cells in each quadrant divide synchronously. The sequence of the quartettes is the same as in the previous generation; but in case of sister cells the larger divides first. To be more specific, the first cells to divide are $a_{1.2}$, $b_{1.2}$, $c_{1.2}$, $d_{1.2}$ and A_3, B_3, C_3, D_3; then, $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, $d^{1.2}$ and a^3, b^3, c^3, d^3; $a^{1.1.2}$, $b^{1.1.2}$, $c^{1.1.2}$, $d^{1.1.2}$; $a^{2.1}$, $b^{2.1}$, $c^{2.1}$, $d^{2.1}$; $a^{2.2}$, $b^{2.2}$, $c^{2.2}$, $d^{2.2}$ (cf. Figs. 93–97).

The result of these divisions is a 64-cell stage, which is like that of *Amphitrite* and *Clymenella*, except that it is more regular ; so regular, in fact, that there is no way of distinguishing one quadrant from another. It is easy to distinguish the two poles and thus the egg axis, but not the sagittal plane of the embryo. One can distinguish from one another the component cells of a quadrant, but cannot say to which quadrant they belong. This is true throughout the history of the cleavage, as far as I have studied it.

The apical rosette is formed just as in *Amphitrite*, *Clymenella*, etc., though the origin of the cells is peculiar in that they lie at first in the segmentation cavity but later elongate and reach the surface (Fig. 98). Since the polar globules may be in any of the cross cells, they are sometimes found far removed from their original position (p. 9, Figs. 98, 100, 101, 104).

At the vegetative pole the eight lower cells, A_4, B_4, C_4, D_4 and a^4, b^4, c^4, d^4, form what may be called provisionally the entoderm plate. These do not divide again at the surface, but become elongated, and their nuclei migrate inward like those of the mesoderm and entoderm cells of *Amphitrite* (Figs. 102, 103).

It is interesting to notice the contrast between the divisions of the animal and the vegetative-pole cells in this last cleavage. The two cell-groups in question were of about the same size and similarly arranged, four cells at the animal pole and four at the vegetative pole. All the cells of both groups divide in the same direction and at the same time, but at the animal pole the result is eight cells, of which the four central ones are the smaller, while at the vegetative pole the result is exactly the reverse — the four central cells are the larger. The relative position of the cells at the two poles of the egg can be seen in Fig. 95.

At the 64-cell stage the living egg shows distinctly the rosette, undivided cross cells, and the prototrochal cilia borne, in part at least, by the cells corresponding to the primary prototroch in *Amphitrite* (Fig. 97, shaded cells).

The situation of the fully formed prototroch and the size and position of its component cells sustain the interpretation

that the primary prototroch is the same as in *Amphitrite* and *Clymenella*. Figs. 100, 101 show that the further divisions of the cross cells correspond in direction exactly to those in *Amphitrite*, while other ectoderm cells conform to the law of alternating oblique cleavage.

I have been unable to discover the origin of the mesoderm in *Lepidonotus;* it may come from d^4 as in other forms, but d^4 cannot be distinguished from a^4, b^4, and c^4, and all of these cells invaginate together with A_4, B_4, C_4, D_4. In the typical

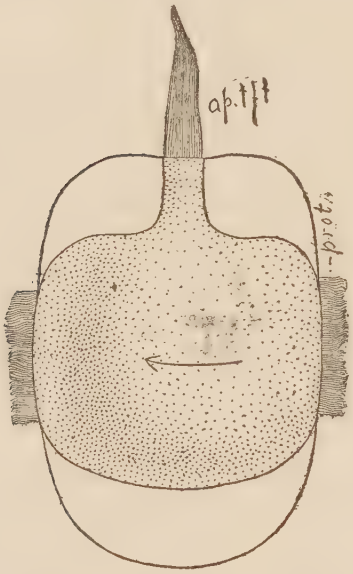

FIG. XIX. — *Lepidonotus*, very young trochophore; *ap.tft.*, apical tuft of cilia; *prot.*, prototroch; the arrow indicates the direction of rotation.

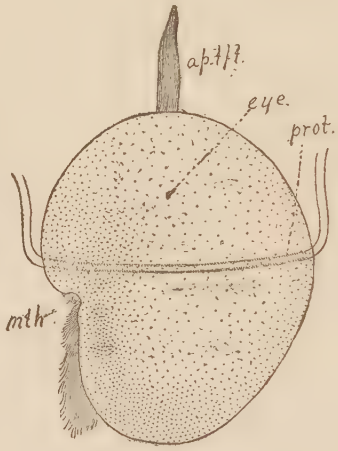

FIG. XX. — *Lepidonotus*, left side, second day; *ap.tft.*, apical tuft; *prot.*, prototroch; *mth.*, mouth.

gastrula (Fig. 104), between the entoderm and ectoderm, cells are sometimes found which are apparently mesoblastic in origin. During the gastrula stage the trochophore assumes a remarkable form, seen in text Fig. XIX. The membrane stands out from the body except at the regions of the prototroch and apical tuft. A much later larva is shown in text Fig. XX — a form maintained for several days.

Influence of temperature, direct heat, and light. — The influence of the temperature of the water upon the rapidity of the cleavage processes is very pronounced. The difference of

a few degrees between the first and the last of the breeding season appreciably increases the rate of development. If artificial heat is applied, even a sudden rise of many degrees does not have the slightest effect on the manner of development, but greatly increases the rapidity. Of several hundred eggs fertilized at 8 A.M. on May 1, the larger number were in the 2-cell stage at 11.30 A.M., though some of them were dividing into four. The eggs were then separated into two dishes. The temperature in dish *A* was kept at that of the sea, 8° C. The other dish, *B*, was placed in a warm bath, and at 11.40 the temperature had risen to 35° C. It rapidly fell to 21°, and there remained. In dish *A* the development continued very slowly. In dish *B*, where the temperature was raised 17° in ten minutes, the eggs took a sudden start and developed twice as quickly as those in the cold water.

Dish *A*, 8° C.	Dish *B*, 21° C.
11.40 A.M. 2– 4 cells, . .	2– 4 cells.
12.07 P.M. 2– 4 " . .	4– 8 "
1.05 " 4– 8 " . .	16–32 "
3.35 " 8–16 " . .	32–64 "
4.00 " 16+ " . .	64+ " (swimming).
4.10 " —— " . .	swimming rapidly.

The reaction of larvæ to light varies with age. When they first begin to swim, they are positively heliotropic, but later, when they are about fourteen hours old (text Fig. XX), most of them are negatively heliotropic. From twenty to about forty hours after fertilization they are apparently indifferent to light, and are scattered evenly throughout the water. At about forty-four hours they exhibit a very marked positive heliotropism, which continues for several days at least.

Larvæ three days old which were strongly heliotropic, crowding to the sides of the dish nearest the window, could not be induced to abandon this position by direct heat rays acting opposite to, at right angles to, or coincident with, the light rays.

The eggs of *Harmothoë sp.* are slightly smaller and clearer than those of *Lepidonotus*, and develop in almost exactly the same manner. I have tried to obtain hybrids between these species, but without success.

D. SCOLECOLEPIS VIRIDIS VERRILL.

The breeding season of this species is nearly over by the first of May. The eggs are deposited inside the sand-tubes in which the females live. They are of medium size, and of bright yellow color. The membrane does not adhere so closely as in some other species (Fig. 105). The first division is unequal. At the next division, the smaller cell very often divides first. In the 4-cell stage *D* has a relative size even larger than usual. The four cells divide almost synchronously into eight, in the usual right-oblique direction. The peculiarity of the 8-cell stage is that the apical cells, a_1, b_1, c_1, d_1, are very small and perfectly transparent, while the vegetative cells are very large and perfectly opaque.

The four lower cells divide again, and the lower products of this division, A_2, B_2, C_2, D_2, are all opaque. Of the four upper products, a^2, b^2, and c^2 are transparent and comparatively small, while d^2 (somatoblast in other forms) is opaque and of enormous size. Very soon the four apical cells, a^1, b^1, c^1, d^1, divide in the usual left-oblique direction (Figs. 110–113).

Though all the previous divisions and the present positions of the cells are the same as in *Lepidonotus* at the 16-cell stage, the relative size of the blastomeres presents a remarkable contrast (Fig. 112). The cells colored brown in Figs. 112 and 114 correspond in origin to the primary trochoblasts of other forms, — *Amphitrite*, *Clymenella*, and *Nereis*.

Notwithstanding the great differences in the size and in the constitution of these sixteen cells, they all divide again almost simultaneously, with the exception of the trochoblasts $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, $d^{1.2}$, the direction alternating as usual with that of the previous division. The failure of the primary trochoblasts to divide with the other cells prevents the egg from actually attaining the typical 32-cell stage. The difference between the relative size of the cells of the *d* quadrant and that of the corre-

sponding cells of the other quadrants is greater in *Scolecolepis* than in any other form I have studied, Fig. 114 (shaded nuclei). For example, $d^{2.1}$ is the largest cell in the egg, $a^{2.1}$, $b^{2.1}$, $c^{2.1}$ are the smallest even more minute than the primary trochoblasts. They correspond in origin to the *secondary trochoblasts in Amphitrite and Clymenella.* This diminutive size of the primary and secondary trochoblasts is significant in view of the suppression of the trochophore in this form.

Owing to the lack of material, I made only the following observations: an apical rosette is formed in the ordinary manner; a typical gastrula stage with elongated blastopore is present somewhat later; the elongated trochophore has a weak prototroch and paratroch; the head segment is composed of clear cells, and contains four unicellular mucous glands lying in a row in front of the mouth, while the body segments are filled with yolk and covered with a layer of epithelial cells (Figs. 115, 116).

E. CHÆTOPTERUS PERGAMENTACEUS CUVIER.*

The eggs of this rare annelid were obtained in August, 1894, by cutting open the females, and were artificially fertilized with sperm obtained from the male in the same manner.

The egg, which is about equal in size to that of *Lepidonotus*, remains with the first maturation spindle in the equatorial-plate stage, until the entrance of the sperm, as in *Clymenella.* The male pronucleus reaches a position near the center of the egg without following any constant path, and there awaits the female pronucleus. The latter moves toward it along the radius of the egg which terminates at the polar globules. Before the pronuclei unite, the two centrosomes derived from the sperm center are already far apart, one on either side of the male pronucleus. Their position indicates the direction of the first cleavage spindle, since a line connecting the two centrosomes coincides with the axis of the spindle. This is perpendicular to the copulation path of the pronuclei, *i.e.*, the egg axis. It follows that the horizontal plane in which the cleavage spindle will lie

* For maturation and fecundation, see Mead.[24]

can be predicted at least as soon as the first maturation spindle appears, and before the sperm enters the egg.

It is clear that the determining factor is neither the path of the spermatozoön, for the relation of the latter to the direction of the spindle varies; nor the path of the female pronucleus in approaching the male pronucleus, for the two male centers have already assumed their permanent position.

The first furrow is meridional as usual, and sinks in all around the egg at the same time, as in *Lepidonotus*. During the first cleavage a peculiar lobe is normally formed on the lower hemisphere, first becoming noticeable when the spindle is in the equatorial-plate stage. It is composed very largely of yolk, although protoplasmic rays from the astrospheres run through it to the periphery. Every phase in the development of this lobe bears a constant relation to that of the karyokinetic spindle (Fig. 117, 118, etc.). During the later phases of the karyokinesis, — the reconstitution of the nuclei, — the lobe becomes constricted at its base, and finally, by the time the new nuclei have assumed a spherical form, is completely resorbed.*

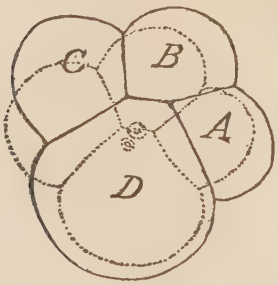

FIG. XXI. — *Chætopterus*, 8-cell stage from lower pole.

The cell *CD*, which bears the lobe, is larger than *AB*. In these two cells the whole karyokinetic process from first to last is carried on simultaneously. The spindles in the two cells are inclined to each other, as in *Amphitrite* (left-oblique cleavage).

The four resulting cells divide simultaneously into eight, in the usual direction. Of the 8-cell stage, the four vegetative cells are, as usual, larger than the four animal cells, and one cell, DD_1, is largest of all (text Fig. XXI). The 8-cell stage of this form is peculiar, however, in that one of the four apical cells, *d*, is much larger than the other three. There are in this stage two

* I said in my previous paper that the lobe was completely constricted off. The statement was based on the study of preserved material which certainly supported this interpretation; but an examination of living eggs plainly shows that I was in error

cross-furrows, one at the animal and one at the vegetative pole, which, instead of being at right angles to each other, are parallel. The eight cells divide in the usual direction and form a 16-cell stage (Figs. 122, 123), in which one of the apical cells $d_{1.v}$, is remarkably large, while its sister cell, $d^{1.1}$, trochoblast, is no larger than $a^{1.1}$, $b^{1.1}$, or $c^{1.1}$. In other respects the 16-cell stage is like that of *Amphitrite* (cf. Figs. 8–122, 11–123).

The nearly synchronous cleavage of all these cells gives us the 32-cell stage (Fig. 124). Every cell is located as in *Amphitrite*, and their relative size is the same with two exceptions: $d^{1.2}$ receives the extra material bequeathed by the cell d_1 of the 8-cell stage, and the four apical cells are of about the same size, — in *Amphitrite* the two dorsal ones are the larger.

Every one of the thirty-two cells divides obliquely to the left, but not synchronously. Already (Fig. 124) three of the cells have spindles, while the others are in the so-called resting stage. The familiar rosette is formed in the typical fashion, and most of the other divisions take place as indicated in the remaining figures by spindles or arrows (Figs. 124–130). On account of the regularity of the cleavage and its resemblance to that of *Amphitrite*, I thought it unnecessary to introduce more figures.

The division of the primary trochoblasts, colored brown, may be seen in Figs. 125–128; the position of the secondary trochoblasts and of $c^{2.1.1.}$ and $c^{2.1.2.}$ in Fig. 132; the mesoderm cells, the above-mentioned rosette, and the primary cross-cells in Figs. 126–131.

An actual 64-cell stage does not occur in *Chætopterus*, owing to the precocious division of certain cells. Soon after the rosette cells are formed, they ingest the polar globules just as do the apical cells of *Lepidonotus* at an earlier period (Figs. 127, 131, 132, p. 266).

I have carried the cell lineage little beyond what may be called the ideal 64-cell stage, but some of the next divisions are of great interest. The primitive cross-cells (colored blue in figures), although in origin and in position exactly like those in *Amphitrite*, divide strictly according to the rule of alternating cleavage, and do not form the cross (Fig. 131). They usually

divide bilaterally (cf. *Amphitrite*, *Clymenella*, *Nereis*, etc.), without exhibiting a trace of the alternating oblique cleavage. Other anomalous divisions occur in the cells corresponding to the primary prototroch of *Amphitrite* and *Clymenella*. Figs. 131, 132 show the cleavage of one of these cells, and I have seen others divide.

Wilson[7] described the larva of this form as having no prototroch. See also Korschelt and Heider.[8] It is possible that there is some correlation between the anomalous behavior of the trochoblasts and the absence of the prototroch.

[7] E. B. Wilson: Observations on the Early Developmental Stages of Some Polychætous Annelids. *Stud. Biol. Lab. Johns Hopkins Univ.*, vol. II, 1882.

[8] Korschelt und Heider: Lehrbuch der Entwicklungsgeschichte.

PART II.—COMPARATIVE AND GENERAL.

In the literature on the embryology of annelids, the record of cell lineage has usually been incidental to the description of the general development of the embryo. Whitman, however, in his well-known work on the embryology of *Clepsine*, 1878, gave a detailed account of the exact origin of those cells which give rise to the mesoderm, nerve-cords, nephridia, etc.; he introduced and proved the value of cell lineage as a method of embryological research.

By far the most important contribution to the cell lineage of annelids since 1878 is that of E. B. Wilson, based on a study of the marine annelid *Nereis limbata*, 1891. He recorded every cell division up to the 58-cell stage, and many of the subsequent cleavages. In the appendix to his paper he briefly describes the cleavage of *Polymnia*, *Spio*, *Aricia*, and *Eupomatus*. Several investigators have shown that the cleavage in certain flatworms and molluscs has many features in common with that in annelids ; the most apparent distinguishing peculiarities are (a) the obliquity of all the early cleavage furrows, and (b) their regular alternation in direction in successive generations of cells. Cleavage characterized by these features may be designated *alternating oblique* (spiral type of Lang, Wilson, and others), and has already been described in the following forms.

Among annelids :

Clepsine, Whitman [9], [10]
Nereis cultrifera, Salensky [11]
Nereis Dummerillii (free-swimming trochophore), Goette [12]
Lumbricus and *Rhynchelmis*, Vejdovsky [13]

[9] Whitman, C. O.: The Embryology of Clepsine. *Quart. Jour. Micr. Sci.*, XVIII, 1878.

[10] Whitman, C. O.: The Germ-Layers in Clepsine. *Journ. of Morph.*, vol. I, 1887.

[11] Salensky, W.: Études sur le Développement des Annélides. *Arch. de Biologie*, III, 1882.

[12] Goette, A.: Abhandlungen zur Entwicklungsgeschichte der Tiere. Leipzig, 1882.

[13] Vejdovsky, Fr.: Entwickelungsgeschichtliche Untersuchungen. Prag, 1883.

Eupomatus uncinatus, Hatschek [14]
Nereis Dummerillii (suppressed trochophore), von Wistinghausen [5]
Nereis limbata and *megalops*, Wilson [1]
Hydroides sp., "
Polymnia nebulosa, "
Spio fulginosus, "
Aricia fœtida, "
Amphitrite ornata, Mead [25] *
Clymenella torquata, " *
Scolecolepis viridis, " *
Lepidonotus sp., " *
Chætopterus pergamentaceus, Mead.[24] *

Among flatworms :

Discocœlis, Lang.[15]

Among molluscs :

Planorbis, Rabl [16]
Neritina, Blochmann [17]
Crepidula, Conklin [18], [19]
Umbrella, Heymons [2]
Unio, Lillie [8]
Limax, Kofoid [4]
Physa, Crampton [20]
Limnæa, "
Patella, Patten.[21]

[14] Hatschek, B.: Entwicklung der Trochophora von Eupomatus uncinatus Phillippi. *Arb. zool. Inst. Univ. Wien*, Bd. 6.

[15] Lang, Arnold: Die Polycladen, etc. *Fauna und Flora des Golfes von Neapel*, vol. XI, 1884.

[16] Rabl, C.: Ueber die Entwicklung der Tellerschnecke. *Morph. Jahrb.*, vol. V, 1879.

[17] Blochmann: Ueber die Entwicklung von Neritina fluviatilis. *Zeitschr. f. wiss. Zool.*, Bd. 36, 1882.

[18] Conklin, E. G.: Preliminary Note on the Embryology of Crepidula fornicata and Urosalpinx cinerea. *Johns Hopkins Univ. Circ.*, vol. X, no. 88, 1891.

[19] Conklin, E. G.: The Cleavage of the Ovum in Crepidula fornicata. *Zool. Anz.*, Jahrg. 15, 1892.

[20] Crampton, H. E.: Reversal of Cleavage in a Sinistral Gasteropod. *Annals N. Y. Acad. Sci.*, vol. VIII, 1894.

[21] Patten, W.: The Embryology of Patella. *Arb. zool. Inst. Univ. Wien*, Bd. 6.

[24] Mead, A. D.: Some Observations on the Maturation and Fecundation of Chætopterus pergamentaceus Cuvier. *Journ. of Morph.*, vol. X, no. 1.

[25] Mead, A. D.: Preliminary Account of the Cell Lineage of Amphitrite and other Annelids. *Journ. of Morph.*, vol. IX, no. 3.

* Previous chapters.

The following chapters are devoted to a comparison of these examples of the alternating type of cleavage from the point of view of (1) *homology of cells*, (2) *developmental mechanics*, and (3) *axial relationships of the embryos.*

I. Homology of Cleavage Cells.

The importance of the similarity in the cleavage of certain annelids, molluscs, etc., has not been unduly emphasized even by those who have worked on these forms, and has been decidedly underestimated by those who have based their interpretation of cleavage upon the echinoderm and the vertebrate egg. Since my own observations tend to enhance the significance of these similarities, I have been led to make the homology of cells a standpoint for comparison,

Essential similarity in origin and fate will be considered a sufficient criterion of the homology of cells, as it is, by common consent, of tissues and organs. That the cleavage cells in the forms under consideration are similar in origin is obvious, and is implied by the fact that they constitute a well-defined type of cleavage; but whether they are alike in destiny is more difficult to ascertain.

The products of the two divisions of the oöcytes — the polar globules — correspond in origin and destiny. The cleavage proper may be "equal" or "unequal."

a. *Equal cleavage.*

Hatschek followed the equal cleavage of *Eupomatus* in detail to sixteen cells, and Wilson describes that of the related *Hydroides* to thirty cells. The observations on *Lepidonotus* recorded in the previous chapters agree precisely with these, and have been extended to the later cleavage.

The first cleavage furrow is always vertical, but in unequal cleavage the two resulting cells vary more or less in size. The larger size of one product is a means of orientation, and since in all cases it possesses certain potential peculiarities, it may be considered in a general way homologous

among eggs of this type. Is the larger size of the cell the cause of its peculiar destiny (according to the law of progressive differentiation), or is the peculiar destiny the cause of its larger size (precocious segregation of embryonic material and specific cell organization)? The answer to this question depends upon *whether one of the two cells in equal cleavage is homologous with the larger cell in unequal cleavage.* This is the immediate problem to be solved by the study of equal cleavage.

In this type the two blastomeres divide into four cells of equal size, the anlagen of the four quadrants. The latter cannot, however, be distinguished from one another at any time during the cleavage, so perfect is the quadriradial symmetry.

Up to the 64-cell stage *Lepidonotus* agrees with *Amphitrite* in the origin and position of every cell: the rosette is always formed in the same manner; the original cross cells proceed to divide in the same peculiar bilateral fashion; the same eight vegetative-pole cells invaginate (some divide again on the surface in *Amphitrite*); the primary prototroch is differentiated at the same time in both forms, and probably from the same cells. *These characters prove beyond question a very high degree of differentiation in the trochophore of Lepidonotus at the 64-cell stage,* even though the quadrants cannot be distinguished from one another. The differentiation from pole to pole is shown by numerous features, — rosette, prototroch, etc.; the bilateral differentiation is proved by the characteristic cross; but the question remains, *which of two planes is the sagittal plane of the embryo?* or, *which of four quadrants is the dorsal one?*

A clue to the solution of the problem is found in forms like *Amphitrite,* where the dorsal quadrant is distinguished not only by the size of its cells, but, after the 64-cell stage, by their characteristic manner of dividing; for example, the mesoderm cell, d^4, always divides bilaterally, and gives rise to the paired mesoderm bands. Unfortunately, it has never been seen to divide in *Lepidonotus* or any other annelid with *equal* cleavage. Again, in *Amphitrite* and *Clymenella,* the interruption in the prototroch is due to the failure of certain cells in the dorsal quadrant to develop cilia, as do the corresponding cells in the

other three quadrants. Now in *Lepidonotus* there is a similar dorsal interruption, and, moreover, the primary prototroch is differentiated at the same time and probably in the same manner as in *Amphitrite* and *Clymenella* (unequal type). If the dorsal interruption in *Lepidonotus* (equal type) arises in the same manner, there must be here also an early differentiation in this quadrant which does not manifest itself in the size of the cells. — But the origin of the *complete* prototroch, like that of the mesoderm, has yet to be ascertained in eggs with equal cleavage. The ascertainment of the origin of one or both of these structures is within reach, and should settle the question whether one of the two blastomeres in equal cleavage is homologous with the larger in unequal cleavage, and the solution of the last question would in turn throw light on the meaning of cleavage and the nature of differentiation, morphological and physiological.

As the matter stands at present, one of two things can be said of the differentiation of the egg of *Lepidonotus* in the 64-cell stage. It has either a complete bilateral organization which is not discernible, or *two* planes of symmetry with four possibilities of orientation, from which it must select one before development proceeds much farther.

b. *Unequal cleavage.*

Eggs with unequal cleavage are characterized by a 4-cell stage with one cell of predominant size. This cell is the same in origin except perhaps in one or two cases.*

The destiny of the other three cells is in most cases very imperfectly known. But the general homology of these early blastomeres is best considered while comparing their more highly differentiated products.

All eggs of this type pass through a characteristic 8-cell

* In Vejdovsky's account of *Rhynchelmis*,[18] the largest cell does not correspond in origin to that in the other forms, but this point needs reinvestigation (cf. Whitman's criticism,[10] p. 126).

In *Physa* (Crampton [20]) the large cell has a different origin, but has the same general fate. It always forms the mesoderm, and at least the larger part of the trunk ectoderm.

stage, consisting of four smaller cells at the animal pole alternating with four larger ones at the vegetative pole. The destiny of these cells in all well-ascertained cases is the same; the four smaller ones together give rise to the umbrellar region of the trochophore, the four lower cells to the subumbrella. When the cells of the upper quartette are comparatively large and divide readily, the umbrella is large and the trochophore active. When, on the other hand, the cells of the upper quartette are smaller and divide less rapidly, the umbrella is smaller and the trochophore less active. *Lepidonotus*, *Amphitrite*, *Nereis limbata*, *Clymenella*, *Nereis Dummerillii*, *Rhynchelmis*, and *Clepsine* form a series of annelids in which there is a gradual decrease in the relative size and karyokinetic activity of the four upper cells, and a corresponding decrease in the size of the umbrella and the activity of the trochophore. The molluscs might be arranged in a similar series, *e.g.*, *Patella*, *Crepidula*, *Unio*, and *Umbrella*.

In the *16-cell stage* the ultimate destiny of four cells, those of the second quartette from the animal pole (trochoblasts), has been completely worked out in *Amphitrite* and *Clymenella*. These give rise to the *primary prototroch* in exactly the same manner in both forms.* Though the worms belong to rather distantly related families (Maldanidæ and Terebellidæ), and the eggs are different in size, in the quantity and quality of yolk, and in the relative size of the trochoblasts, yet the latter agree perfectly in mode of origin and in destiny, and must be placed in the category of homologous cells. The observations in other forms, so far as they have been extended, bear out this homology.—In *Lepidonotus* the divisions of the trochoblasts take place exactly as in *Amphitrite* and *Clymenella*, and when they are completed the trochophore almost immediately begins to swim. The prototrochal cilia are produced, in part at least, by these cells, and a complete correspondence is very probable.

Scolecolepis contrasts sharply with *Lepidonotus*, since it has an almost completely suppressed trochophore. The upper hemisphere, umbrella, is itself very small, while the trochoblasts (?)

* These two annelids are the only ones in which the history of *all the products* of the trochoblasts has been completely worked out.

are conspicuous for their diminutive size and their tardiness in dividing, — just what we should expect of trochoblasts in a suppressed trochophore, if the homology could be extended to this form. Unfortunately, the further history of these cells is unknown.

In *Nereis*, according to Wilson,[1] only twelve of the products of the trochoblasts enter the prototroch, so that the (primary) prototroch consists of four groups of *three* cells each instead of four groups of *four* cells each, as in the case of *Amphitrite* and *Clymenella*. Thus the homology would appear to be somewhat imperfect, but it is sustained by a closer comparison of the behavior of the trochoblasts in *Nereis* with those in *Lepidonotus*, *Amphitrite*, and *Clymenella*. In each, the four trochoblasts arise in the same manner and divide obliquely to the right. In the first three annelids the eight resulting cells again divide obliquely (to the left), while in *Nereis*, according to Wilson, the direction alternates, one cell dividing horizontally, the next vertically, and so on around the egg. It will be readily admitted that *Lepidonotus* and *Amphitrite* have a more typical cleavage than *Nereis*, for they have less yolk and the cleavage is more regular. If we compare the figures of *Nereis* with those of the more regular forms, the so-called horizontal and vertical cleavages show at least a reminiscence of obliquity. In *Nereis* the four products of the trochoblasts which lie nearest the animal pole are said not to enter the prototroch ; in *Amphitrite* and *Clymenella* these cells at first have a similar position, but later certainly form part of the prototroch. In *Nereis*, furthermore, the four cells in question have not been seen to divide again, and we do not know how the prototroch is completed.

I think these facts warrant the assumption that the history of the trochoblasts is the same in *Nereis* as in the other forms.

In *Chætopterus* the trochoblasts divide into sixteen cells in the regular way, but some of them *divide again*, — a fact which is significant, because *Chætopterus* is said to have no prototroch.

Somatoblast. — One other cell in the 16-cell stage, the somatoblast (d^2), is already distinguished from the others by the fact that it contains the anlage of the whole or the greater part of the trunk ectoderm in all annelids where it has been

worked out — *Clepsine*, *Rhynchelmis*, *Nereis Dummerillii*, *Nereis limbata*, and *Amphitrite*, and also in molluscs (cf. Lillie,[3] p. 25). Whether the individual cells of the somatic plate (descendants of the somatoblasts) are homologous in different animals cannot be discussed profitably until we have more data. The only products of the somatoblast whose *exact* origin and destiny are known are, I believe, the teloblasts (neuroblasts and nephroblasts) in *Clepsine* and the paratroch cells in *Amphitrite*. Wilson considers certain of its products in *Nereis* to be without doubt the homologues of the neuro-nephroblasts in *Clepsine*; but it seems to me that the ground for such an homology is extremely insecure (p. 250).

The relationships of the other cells can be more clearly understood by comparing their more highly differentiated products at the 32 or at the 64-cell stage.

32-cell stage, the secondary trochoblasts. — In annelids with a free-swimming trochophore, *Lepidonotus*, *Amphitrite*, *Clymenella*, *Nereis limbata*, etc., the sixteen cells divide at almost the same time into thirty-two cells; they always have the same relative position, forming eight quartettes which alternate from pole to pole. The upper sixteen cells constitute the umbrella, the lower sixteen the subumbrella. a^2, b^2, c^2, and d^2, each divides obliquely into two cells, and it is the destiny of the upper products of the first three ($a^{2.1}$, $b^{2.1}$, and $c^{2.1}$) which now interests us. The cleavage of these secondary trochoblasts has been followed in detail only in *Amphitrite* and *Clymenella*, but in these forms their behavior is similar to an extraordinary degree. The divisions are the same in all three quadrants: each secondary trochoblast divides into four — three large cells and one which is very minute. The former soon acquire cilia and form part of the prototroch, while the minute cell does not become ciliated, and later divides again. A more impressive example of *precise similarity in origin and destiny of cleavage cells* in very late stages could hardly be imagined.

The cleavage of *Scolecolepis*, as far as it has been followed, sustains this homology of the secondary (as of the primary) trochoblasts, for the three secondary trochoblasts ($a^{2.1}$, $b^{2.1}$, and

$c^{2.1}$, Fig. 114) are even more diminutive than the primary, and diminutive trochoblasts are to be expected in suppressed trochophores, on the principle of the homology of cleavage cells.

Wilson ascribes a very different fate to these three cells in *Nereis*. They arise in the same way as in *Amphitrite* and *Clymenella*, and undergo one division in the same manner (the next divisions are not recorded); but two of the cells instead of contributing to the prototroch form the "latero-dorsal region of the trunk" (*l.d.*, text Fig. IV).

I think, however, that the fate of these cells in *Nereis* requires further confirmation, for the following reasons: (1) in *Amphitrite* and *Clymenella* such a fate of $a^{2.1}$ and $c^{2.1}$ is out of the question, because all but a very minute portion of these cells enters the prototroch, and because the latero-dorsal region, in *Amphitrite* at least, is formed from another cell, the somatoblast d^2; (2) in *Nereis* only one division of the cells is recorded, while the origin of the rest of the prototroch remains unsolved; (3) such a fate of these cells involves a serious discrepancy between the behavior of the somatic plate (ventral plate) cells in *Nereis* and in the other forms, while in *Amphitrite* and *Clymenella* — the only forms in which their fate has been accurately ascertained — there is a remarkable agreement.

The stomatoblasts (*Nereis*) *and larval mesoblast* (*Unio*). — In *Nereis* Wilson records the peculiar fate of the sister cells of the secondary trochoblasts, *i.e.*, of the cells $a^{2.2}$, $b^{2.2}$, $c^{2.2}$. They form a ring about the stomodæum and hence are called *stomatoblasts*. But Lillie shows that in *Unio* $a^{2.2}$ has an equally peculiar fate: it forms the *larval mesoblast*. The inference has been drawn and was explicitly stated by Lillie that in this case cells of the same origin have different destinies, — a blow to cell homology (Lillie,[3] p. 37). Stated in this way, that $a^{2.2}$ is a stomatoblast in *Nereis* and the larval mesoblast in *Unio*, the inference is well warranted. But, if we examine more critically the fate of these cells in both forms, an obvious fallacy appears. Wilson does not describe in detail the cleavage of the cell in question, though he shows that it divides at least once in the same direction as in *Amphitrite* and *Clymenella*. Only one product of

this division becomes the *stomatoblast.* What becomes of the other product? In *Unio* (p. 24, Figs. 42–47) Lillie describes two or three small cells as "budded off" from $a^{2.2}$ before the latter sinks into the interior to form the larval mesoblast. In other words, $a^{2.2}$ *divides into a group of cells, one of which is the actual larval mesoblast.* What becomes of the others?

There is no reason for believing that the product of the division of $a^{2.2}$, which in *Nereis* becomes a stomatoblast, corresponds in origin to that which in *Unio* becomes the larval mesoblast. But there is excellent reason for believing that it does not, for it is the *lower* product of the first division which in *Nereis* forms the stomatoblast, and the *upper* product which in *Unio* gives rise to the larval mesoblast.

The transition to the 64-cell stage. — The rest of the cells of the 32-cell stage have the same origin and position in the various forms under consideration, but the examination of their morphological relations will be facilitated by comparing their products after the next cleavage, namely, at the 64-cell stage. The transition from thirty-two to sixty-four cells is itself of interest when one compares *Nereis* with more regular forms.

In *Lepidonotus*, *Amphitrite*, *Clymenella*, and *Chætopterus* all the thirty-two cells divide in a left-oblique direction, according to the regular rhythm, and, except in *Chætopterus*, so nearly at the same time that an actual 64-cell stage results.

Wilson divides the cleavage of *Nereis* into a 38, a 42, and a 58-cell stage, but the fact that in this annelid the division of the cells is not synchronous need not confuse us. In *Lepidonotus*, *Clymenella*, *Amphitrite*, and *Chætopterus* all thirty-two cells divide in the left-oblique direction, and it would seem from Wilson's figures, that the same thing may be said of *Nereis* (of course excepting A_3, B_3, and C_3, which do not divide at all). Therefore Wilson's statement that the *transition from the "spiral"* (oblique) *to the bilateral type of cleavage occurs at this stage*, and the consequent inferences, may be questioned.

The 64-cell stage. — We will begin our comparison with the cells at the animal pole. The *apical rosette*, consisting of four very small cells, arises in exactly the same way in *Nereis*, *Spio*,

Aricia, *Polymnia*, *Amphitrite*, *Clymenella*, *Chætopterus*, and *Lepidonotus*. Conklin finds a similar rosette in the mollusc *Crepidula*, and Heymons mentions it in *Umbrella*, but gives no figure. Lillie did not follow the cleavage of the apical cells in *Unio* far enough to find out whether the rosette existed or not. In *Amphitrite* and *Clymenella* all four rosette cells divide again in the regular reverse oblique direction, and Wilson figures one of these cells as dividing in this way in *Nereis*. Their division in other forms has not been recorded. In *Lepidonotus* it is certain, and in *Amphitrite* and *Nereis* extremely probable, that the rosette cells, or their products, bear the cilia of the apical tuft. The rosette cells, therefore, seem to have the same origin and fate.

The four cells which alternate with the four of the rosette are the parent cells of the *cross*, a peculiar pattern first described by Wilson[1] in *Nereis*, *Polymnia*, and *Aricia*. I have found an exactly similar cross in *Amphitrite*, *Clymenella*, and *Lepidonotus*. The cleavage of the four parent cells departs so abruptly from the method of the earlier cleavage, and is so similar in all annelids where the cross occurs, that it deserves special mention. The cross has been described in representatives of seven genera belonging to six different families; in *Spio*, *Polymnia*, and *Aricia* without figures, so that we will confine our comparisons to *Nereis*, *Lepidonotus*, *Amphitrite*, and *Clymenella*. In all of these the four parent cells arise in the same way and lie symmetrically, two on either side of the middle line, one in each quadrant. Each divides horizontally and bilaterally, so that the pattern on one side is the counterpart of that on the other, and I have searched in vain for any reminiscence of oblique cleavage. The outermost cells in each arm soon divide again in the same direction, making three cells in a row.

Peculiar interest attaches to the middle cells of the dorsal arms, because their destiny has been ascertained in *Nereis* and *Amphitrite*. In *Nereis* they do not divide again, but sink into the interior of the egg and become what Wilson provisionally calls "head-kidneys." In *Amphitrite* also they sink beneath the surface, except for a slender stalk, and form a pair of immense unicellular mucous glands. Although

the function of these cells in *Nereis* is not definitely known, they are doubtless homologous with those in *Amphitrite*, since they are the outcome of such peculiar and exactly similar cleavages, and do not divide again, but become specialized unicellular organs. In other forms we know nothing of the ultimate destiny of these cells; they have never been observed to divide and they vary in size, being quite small in *Clymenella.*

The middle cells of the ventral (anterior) arms divide meridionally both in *Nereis* and in *Amphitrite.* In *Nereis* we know nothing further of their destiny, but in *Amphitrite* they show a decided tendency to sink below the surface like the cells of the dorsal arms; and, although I have not followed their history, it is certain that they correspond exactly in *position* to two pairs of ventral unicellular mucous glands.*

Several further divisions of the cross have been ascertained in *Nereis*, *Amphitrite*, *Clymenella*, and *Lepidonotus*. As far as known, the divisions are always strictly bilateral, in the same direction, and preserve their tendency towards quadriradial symmetry in all four quadrants; but the pattern of the cross becomes indistinguishable after a few divisions.

Wilson says that in *Nereis* "there can be no doubt that the cross gives rise in large part to the cerebral ganglion." It would appear from its position in *Amphitrite* that it does contribute to the formation of the ganglion, but I think that in both cases the fate of the cross cells, excepting, of course, the head-kidney and mucous glands above referred to, is doubtful.

We do not know the particular fate of the cells which lie between the arms of the cross, though in *Amphitrite* some of those lying in the dorsal quadrant pass through the interruption

* If the inference that these cells become the mucous glands is correct, indeed, if it is not, we have here a good illustration of what may be called a quadriradial symmetry in the trochophore, especially in the umbrella; a symmetry by virtue of which phenomena occurring in one place, not only repeat themselves on the opposite side of the sagittal plane, but in each of the other three quadrants. This tendency is traceable in the cleavage in the umbrellar region and in the distribution of the purely larval organs, prototroch, mucous glands, etc. In a form like *Lepidonotus*, the quadriradial symmetry is apparently perfect, but in forms with unequal cleavage it appears to be gradually giving way to the strictly bilateral type as illustrated by the series *Amphitrite*, *Nereis limbata*, *Clymenella*, and *Nereis Dummerillii.*

of the prototroch, and contribute to the ectoderm of the subumbrella (p. 239).

The cells of the prototroch have already been considered, and this brings us to the lower hemisphere, or subumbrella. While the somatic plate as a whole has a similar origin and fate in the various annelids, we have not data enough to determine whether the homology may be extended to the individual cells. It seems probable that the "neuroblasts" and "nephroblasts" of *Clepsine*, *Rhynchelmis*, and *Lumbricus* are homologous.

Wilson believes, moreover, that the *proteloblasts* in *Nereis*, are the homologues of the *neuro-nephroblasts* in *Clepsine*, but I find nothing in *Amphitrite* to support this view.

The early divisions of the cells of the somatic plate have a striking similarity in the various forms. One cell in particular is conspicuous for its similarity in *Nereis*, *Amphitrite*, *Clymenella*, and *Unio*. It lies in the lower right-hand corner of the somatic plate (marked $x^{1,2}$): it gives rise to part of the proctodæum in *Amphitrite*. A comparison of the exact origin of the paratroch in the various forms would be of greatest interest.

The secondary trochoblasts have been discussed. As to the rest of the ectoderm on the lower hemisphere, we know only the general fate of groups of cells. In *Nereis* Wilson says that the *terminal cells* which form the proctodæum are "certainly in part the offspring of the primary mesoblast," but this cannot be said of *Amphitrite*.

We have records of the origin of the mesoderm in the following forms: among annelids, *Clepsine*, *Nereis Dummerillii* (free-swimming and suppressed trochophore), *Nereis limbata*, *Spio*, *Aricia*, *Polymnia*, *Amphitrite*; among molluscs, *Neritina*, *Planorbis*, *Umbrella*, *Unio*, and *Crepidula*; among polyclades, *Discocœlis*. A comparison of the mesoblast formation in these forms yields additional evidence for the homology of cleavage cells. In all except *Clepsine*, *Nereis Dummerillii*, and *Discocœlis*, the original mesoderm cell arises from the same one of the fourth generation of cells, which has descended from *D* of the 4-cell stage. *The unpaired mesoderm cell belongs to the ideal 64-cell stage, and is one of the second quartette of cells counting from the vegetative pole, and always arises by a left-oblique*

cleavage. The next furrow always divides this cell equally into a right and a left mesoderm cell.

Clepsine, as described by Whitman,[10] fails to accord with the above in that the original mesoderm cell is differentiated at the ideal 16-cell stage instead of at the 64-cell stage, but Dr. Whitman tells me that certain small cells are budded off before the bilateral division of the mesoblast. It is possible, therefore, that the origin of the mesoblast cell in *Clepsine* is not exceptional.

I fully concur with Wilson in his criticism of the origin of the mesoblast in *Nereis Dummerillii*, as recorded by Goette and von Wistinghausen. Goette's[12] account of the cleavage is incomplete, and von Wistinghausen[5] probably overlooked a cell, and thus threw the responsibility of mesoblast formation upon the wrong one (cf. Wilson,[1] p. 435).

According to Lang[15] the origin of the mesoderm in *Discocœlus* differs from that in the annelids and molluscs in at least two important respects: it arises not from one, but from all four quadrants, and from cells of two generations, both earlier than that giving rise to mesoderm in the annelids, etc.

In annelids these two generations of cells form a part of the ectoderm of the subumbrella, including a portion of the prototroch. Granting, for a moment, the correctness of Lang's observations, we find ourselves in a dilemma well stated by Wilson,[1] p. 448: "Unless, therefore, we are prepared to maintain the absurd proposition that the mesoblast of the polyclade is homologous, not with the mesoblast of the annelid, but with the ectoblast of the lower hemisphere (including, of course, the ventral plate with the ventral nerve cord), we cannot escape the conclusion that exact equivalence of embryological origin is not a proof of homology, so far at least as the cleavage stages are concerned." Again, in his lecture on "The Embryological Criterion of Homology,"[22] Wilson says: "We find (in the polyclade) a cleavage very closely resembling the annelid type in form, yet the individual blastomeres have from the very start an entirely different morphological value." Lillie[3] (p. 37) mentions the mesoblast of *Discocœlis* as affording another instance of cells of different origin which have the same destiny.

However, I am not convinced that the cells described by Lang do give rise to the mesoderm, and I believe it possible that the mesoderm is formed in the same manner and from exactly the same cell as in the annelids with unequal cleavage.

Lang figures the cleavage up to about the 64-cell stage. At this time the cells which, according to his description, become the mesoderm, lie upon the surface, and even extend slightly over the ectoderm. To determine the exact cell lineage of any organ requires a close and uninterrupted series of observations. The stages from 64 cells to the end of gastrulation, are, of course, the critical stages for establishing the origin of the mesoderm. Lang does not record, either in text or in figures, any observations covering this important period.

We now naturally turn our attention to those cells in *Discocœlis*, which in other animals, with this type of cleavage, regularly give rise to the mesoderm bands. Lang's Fig. 12, Plate XXXV, of the lower pole of *Discocœlis* corresponds in every essential point to the ideal 32-cell stage of annelids and molluscs with unequal cleavage. All the cells have the same origin and relative position, and belong to the same generation. The four vegetative cells are comparatively large, and one exceeds the others in size. Now in the annelids these four cells regularly divide upon the surface; the eight resulting cells are of the same generation and lie in a characteristic position, four meeting at the vegetative pole and four alternating with them. One of the latter, the offspring of the *larger cell* (*d* quadrant), is always the mesoderm cell, and lies in the *middle line of the future body*, while the other seven are entodermal. In *Nereis* only the larger cell divides on the surface, so that the entoderm has but four instead of seven cells; a fact which only emphasizes the significance of the division giving rise to the mesoderm cell. In all forms where its origin is known, the mesoderm cell begins at once to behave in a perfectly unique and characteristic way. — It always divides into two equal cells, one of which lies on the right and one on the left of the middle line. These cells give rise to the right and left mesoderm bands respectively.

When Lang's figures and text are examined in the light of this comparison, the correspondence of *Discocœlis* to the other forms is complete in every detail: so complete as to be fairly startling. The eight cells are formed in the same manner (Figs. 12–16). The one which corresponds to the mesoderm cell in the other animals, divides bilaterally, and one product lies on either side of the middle line (Fig. 17, a_1, a_2, Pl. XXXV, text Fig. XXV, p. 340).

Since in a large series of forms the mesoderm cell is the same in origin (same generation and position), and immediately divides in a manner different from all the other cells, and since a cell in *Discocœlis* corresponds exactly in origin and begins the same characteristic career, I believe it may be the mesoderm cell in *Discocœlis* also.

In many cases the paired mesoderm cells divide while still at the surface. The ventral or "anterior" products, whose exact fate is uncertain, may be as large as the posterior (*Clymenella*), or much smaller, as is usually the case (*Nereis, Unio, Umbrella, Spio, Aricia, Crepidula*). In *Spio* and *Aricia* Wilson says the anterior products "are very minute and appear to be rudimentary." In *Amphitrite* they are extremely minute, arising at a later period by a cleavage which seems to defy mechanical conditions, and are carried into the segmentation cavity and lie at the anterior ends of the mesoderm bands. In *Polymnia* this "preliminary superficial budding seems not to take place."

We cannot interpret this peculiar division until more is learned about the exact fate of these cells. The fact that they are sometimes of large size and divide readily, while at other times they are much smaller or exceedingly minute and are slow in dividing, would seem to indicate that they are the anlage of some variable or rudimentary structure.

Entoderm. — The cytogenetic origin of the entoderm is another example of cell homology. In many forms the larger of the four cells at the vegetative pole in the 32-cell stage divides, the outermost product giving rise to the *mesoderm cell*, while the rest of the products are *entodermal*, whether they divide again on the surface or not. In *Nereis* the entoderm

plate is composed of four cells: in other forms three of these divide on the surface and give rise to an entoderm plate of seven cells. This appears to be the case in the molluscs, *Planorbis*, *Neritina*, and *Umbrella*. In *Lepidonotus* the same number of divisions obtains, but we do not know how many or what cells are mesodermal. In several annelids, *Polymnia*, *Aricia*, *Amphitrite*, *Clymenella*, the four inner cells of the plate divide again, making the entoderm plate number eleven cells.

The ova of polychætous annelids, and of many other forms in related phyla pass through cleavage stages easily referable to a common type; the regularity and topographical peculiarities of these stages have made it possible to compare the corresponding cells among the various animals in all stages of cleavage, while the very early differentiations of the blastomeres often makes this comparison of paramount theoretical value. The more extensive and thorough the comparison, the better warranted the inference that there exists between the cleavage cells of various forms the same morphological relationship which exists between adult organs and tissues, *i.e.*, *homology*.

The main objections urged against cell homology in this type of cleavage are the alleged difference in the fate of the cell $a^{2.2}$ in *Nereis* and in *Unio*, and the difference in origin of the mesoblast in *Discocœlis* and in the other forms. On closer scrutiny, these objections lose their force because of a seeming oversight in the first instance, and the lack of evidence in the second.

In attempting to explain annelid cleavage by means of the mechanical principles already deduced from experimental sources and applied with varying success to other forms of cleavage, two points present themselves: first, in the echinoderms, vertebrates, etc., very little has been ascertained concerning the normal relations between the cleavage cells and the organs and tissues of the adult of the same individual, and between the corresponding cleavage cells of different species; second, in the annelids, etc., while considerable is known concerning both of these normal relations, no successful experiments have been

made which enable us to analyze the causes of these relations into mechanical components. What is to be said at present about these components must be deduced from a comparison of normal cleavage in different forms; often where cleavage *processes are similar*, the mechanical *conditions are different;* and, where the *conditions are similar*, the *processes are different.*

II. Cleavage Considered from the Point of View of Developmental Mechanics.

The doctrine of the mechanical causes of organic forms must, when applied to annelid cleavage, account for (1) the relative size of the cells, (2) the direction of the cleavage, and (3) the time or rate of cleavage, for all three are potent factors in determining the specific form of the trochophore.

Relative size of the cells.— Aside from the very unequal cleavage of the *oöcytes* in the formation of the polar globules, the first cleavage of the egg itself is sometimes equal, sometimes unequal. The explanation has been offered that the inequality is due to the influence of yolk; that the karyokinetic apparatus is not able to effect a cleavage through the middle in cases where the yolk is abundant. A comparison of *Lepidonotus*, which has little yolk and equal cleavage, with *Amphitrite* and *Clymenella*, which have more yolk and unequal cleavage, seems to sustain this explanation; but a further comparison with other forms shows that the influence of yolk cannot be considered the chief determining factor of the relative size of cells. The egg of *Chætopterus*, for instance, is about the size of that of *Lepidonotus* and has apparently the same amount of yolk, yet it divides very unequally. The eggs of *Crepidula* have a very large amount of yolk and are much larger than *Unio*, yet the first cleavage of the former is nearly equal, that of the latter very unequal. In the later cleavage stages, cells with a large proportion of yolk are as likely to divide equally as unequally. Examples are numerous. In *Amphitrite* the smaller of the first two blastomeres, though it arises by an unequal division, divides equally, while each product of this cleavage divides *unequally*. On the other hand, the largest

cell of the 8-cell stage arises by an unequal division, but divides about *equally* (D_2 and d^2, Pl. X). The divisions corresponding to the latter in the other three quadrants are extremely unequal, though the relative amount of yolk in the cells is less. The first division of the original mesoderm cell in *Amphitrite* is equal, the division of each product extremely unequal. In *Clymenella* the original mesoderm cell divides equally, and the daughter cells also.

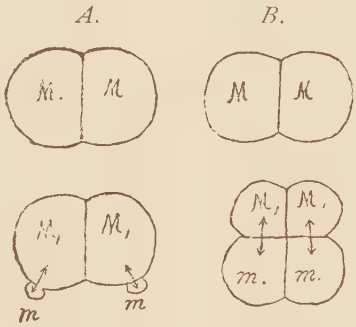

FIG. XXII.—Shows the relative size of the products of the paired mesoblasts, *M* and *M*, after their first division; *A*, *Amphitrite*; *B*, *Clymenella*.

Cells without much yolk divide equally or unequally without regard to size, as may be seen by simply examining the figures of the upper hemisphere in the various eggs at all stages, especially those representing the formation of the rosette, cross, and secondary prototrochal cells in *Amphitrite* and *Clymenella*.

As to the influence of gravity in determining the size of the cells, it needs only to be said that many eggs, *i.e.*, *Amphitrite*, *Clymenella*, and *Nereis*, develop normally whether in one position or another.*

Similarity in *position* of cells does not insure similarity in the relative size of products. In the 32-cell stage of *Lepidonotus*, four cells lie at the animal pole in the same position, with reference to surrounding cells, as the four cells at the vegetative pole. The eight are of about the same size, and divide unequally in the same direction and at the same time; but at the animal pole the resulting central cells are the *smaller* products, while at the vegetative pole the central cells are the *larger* products. In *Discocœlis* the four vegetative cells have the same position as in *Lepidonotus*, but the four central products of the next division are the *smaller*. The division of the paired mesoderm cells and the secondary trochoblasts in *Amphitrite* and *Clymenella* furnish additional illustrations (text Figs. XXII, XXIII).

* Dr. W. M. Wheeler tells me that the same is true of the egg of *Blatta*.

The direction of cleavage. — Mechanical principles which are apparently sufficient to explain the direction of cleavage in one instance are totally inadequate in others. Thus, when we have decided that certain factors determine the horizontal direction of the first cleavage spindle, for example, the principle of equal or least resistance due to the segregation of yolk, these factors must be considerably modified when called upon to explain the *vertical* position of the two maturation spindles; for the conditions, segregation of yolk, etc., are the same.*

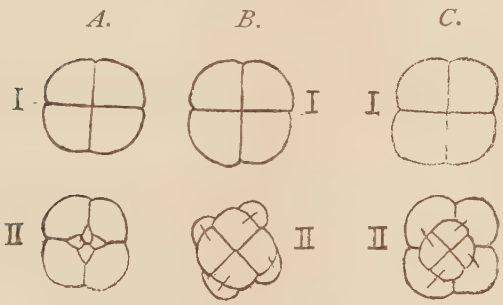

FIG. XXIII. — *A*, *Lepidonotus*: *I*, apical cells; *II*, their products. *B*, *Lepidonotus*: *I*, vegetative-pole cells; *II*, their products. *C*, *Discocœlus*: *I*, vegetative-pole cells; *II*, their products.

The appearance of the peculiar lobe in *Chætopterus*, simultaneously with the first cleavage, seems to indicate that the cleavage does not take place in the direction of *least resistance*, else it would coincide with the axis of the lobe, as in Loeb's [26] experiments on sea-urchin eggs.

In this type of cleavage the spindles of the two blastomeres are inclined to each other, and almost always in the same direction. Why is this? And why does *Physa* form an exception to the rule?

In *Lepidonotus*, *Amphitrite*, and *Clymenella* from the division of the first two blastomeres up to the "ideal" 64-cell stage the regular alternation in the direction of cleavage accords with the "law" that successive cleavage planes tend to lie at right angles.† After the ideal 64-cell stage many of the cells divide

[26] J. LOEB: On Some Facts and Principles of Physiological Morphology. *Biol. Lectures.* Woods Holl, 1893.

* Watasé,[23] in his "Studies on Cephalopods," *Journ. of Morph.*, vol. IV, no. 3, figures an egg of *Loligo* in which the blastoderm is developing on the side instead of on the end of the egg. The pattern of the cleavage is the same as in the normal egg, though the conditions must be decidedly different.

† In the retarded cleavage on the upper hemisphere of *Umbrella* there are certain divisions which do not agree in direction with the corresponding

without the slightest regard to this rule of alternating cleavage. Their only concern seems to be to form a pattern bilaterally symmetrical with respect to the sagittal plane of the embryo. This tendency is well illustrated in the formation of the *cross* in all forms where the latter occurs; in the characteristic first division of the mesoderm cell (d^4), even in *Discocœlis;* and in the further division of the somatic-plate cells (p. 241). These cleavage phenomena present three general features which are to me especially interesting: (1) certain cells divide bilaterally, while their adjacent sister cells continue to divide in the alternating oblique rhythm; (2) the former cells *suddenly* disengaged themselves from the influence, whatever it may be, which compels the parent cells and sister cells to divide in the alternating rhythm; (3) in most cases the cells which so promptly respond to the influence of bilaterality, so to speak, are the anlagen of bilaterally symmetrical structures, *e.g.*, mucous glands (*Amphitrite*), head-kidneys (*Nereis*), mesoderm, somatic plate, etc.

The direction of the first division of the paired mesoblast cells in *Amphitrite* stands in direct contradiction to the "law" that the spindle lies in the direction of least pressure or that it lies in the long axis of the protoplasm of the cell, for it coincides with the direction of greatest pressure, and is at right angles to the long axis (cf. p. 247). Additional importance attaches to this division, for, from the point of view of cell homology, it is a reminiscence of a surface division which still persists in some forms; and thus we have here a contest between the influence of mechanical surroundings and an intrinsic tendency to divide in a certain direction, at a certain time, and the latter prevails.

The "parent cross-cells" in *Chætopterus*, on the one hand, and in *Nereis*, *Lepidonotus*, *Amphitrite*, etc., on the other, afford remarkable examples of cells of similar origin and position dividing in different animals in fundamentally different direc-

division in the above forms. Why these divisions are retarded, and what is the destiny of the cells, are, unfortunately, unsolved problems. It looks, however, as though the direction of the cleavage was influenced by the position of the surrounding cells, and, therefore, may accord fairly well with the mechanical hypothesis.

tions. The alternating oblique direction of the division of these cells in *Chætopterus* compared with the strictly bilateral division in all the other forms makes it doubly difficult to explain either case on mechanical principles.

I have already given my reasons for believing that Wilson is mistaken in asserting that the *bilateral period* in *Nereis* begins with the 38-cell stage. It certainly begins only after the 64-cell stage in the forms I have studied. I disagree further with Wilson[1] when he says that the *cause* of the introduction of bilateral cleavage is the reduction of the "posterior macromere" to the size of its fellow. Bilateral cleavage occurs in *Lepidonotus* and in *Umbrella* where there can be no such "reduction," because the macromeres are equal from the first. Moreover, in *Chætopterus*, where the posterior macromere *is* larger, certain bilateral divisions which are always found in the other forms do not occur.

I do not understand how Kofoid could have inferred from Wilson's or Heymons's figures or text that the rule of alternating (spiral) oblique cleavage holds good throughout the cleavage of *Nereis* up to the "58-cell stage," and of *Umbrella* up to the 91-cell stage. The 58-cell stage in *Nereis* is brought about by the obviously bilateral division of some of the *cross cells* and of the mesoderm cells, while the 91-cell stage in *Umbrella* is attained only by the bilateral division of the mesoblast.

The rate of cleavage. — The absolute rate of cleavage varies with the temperature of the water and among different species, and cannot be predicted from the size or physical appearance of the egg (cf. *Amphitrite* and *Unio*). In all polychætes which I have studied, the division of the oöcytes of the first order, *i.e.*, the formation of the first polar globule, will not take place until the sperm has entered the egg. Just how the entrance of the sperm stimulates the cell to karyokinetic activity is unexplained, but the phenomenon is suggestive in that it shows that, in one case at least, it is not the mass of the egg nor the presence of more or of less yolk that determines the *time* of cell division, but a stimulus of some kind, analogous, perhaps, to that which starts into activity the motor apparatus of pigment cells, leucocytes, or muscle cells.

When the cleavage is once started, it is evident that the form of the embryo is conditioned by the relative rapidity of cell divisions in certain regions, as well as by the relative size of the cells and the direction of cleavage.

A close comparison of the rate of cleavage of corresponding cells in the eggs of various annelids, molluscs, etc., and of the rate in different cells in adjacent areas of the same egg, clearly demonstrates that neither the effect of gravity, the size of the cells, their position, nor all these together are sufficient to account for the rate of cleavage. As to the effect of gravity, it is only necessary to repeat that many eggs develop normally in any position. Let us test the other factors in a number of instances. In *Nereis* the smaller of the two unequal blastomeres regularly divides first ; in *Chætopterus*, on the other hand, though unequal, both blastomeres divide at exactly the same time. I have examined this egg with particular care by means of sections, and find that at successive stages of karyokinesis even the daughter centrosomes at the ends of the spindles are just as far apart in one cell as in the other. In *Amphitrite*, also, the two cells divide at about the same time, — sometimes, however, the larger one is slightly in advance.

In many forms the division of the first eight cells takes place synchronously, whether the cells are of nearly the same size and contain little yolk, as in *Lepidonotus*, *Eupomatus*, etc., or whether, as in many other forms, of which *Scolecolepis* is perhaps the best example, the four upper cells are comparatively small and free from yolk, while the four lower cells are large and full of yolk.

The mesoderm cell, in several forms, manifests great karyokinetic activity, while the entoderm cells, which correspond exactly in origin, but lie in the other quadrants, cease dividing temporarily, — yet in size and amount of yolk the mesoderm cell often very closely resembles those of the entoderm. The cells of the upper hemisphere in *Unio* are tardy in division, but are of moderate size and, to all appearances, similar to the corresponding cells of *Amphitrite*, *Nereis*, etc.

The consideration of the rate of cleavage must include not only cells which are precocious or tardy, or cease to divide tem-

porarily, but also those which suddenly stop dividing altogether. For example, the cells of the primary prototroch in *Amphitrite*, *Clymenella* and *Nereis;* the secondary prototroch cells in *Amphitrite* and *Clymenella;* the "head-kidneys" of *Nereis;* the mucous glands and paratroch cells in *Amphitrite.* These cells possess no peculiarity of size, yolk segregation, nor position, which can account for their sudden disinclination to divide. The neighboring cells, which continue to divide, all share the same characteristics, as far as size and yolk are concerned; and, as for position, in *Chætopterus* the "prototrochal cells" *do* divide again, — at least some of them.

The conceptions of the mechanical interaction of cleavage cells as units of mass are too crude to offer any explanation of these complex phenomena.

The cytogenetic origin of the prototroch and paratroch forms an interesting study in cell differentiation, since these organs are of great morphological significance and high physiological specialization, and since their exact origin cell by cell from the ovum has been accurately ascertained. Thus, for example, if we apply a "theory of determinants" to these cases, it would appear that the determinants of the *primary prototroch* are all segregated in the four "trochoblasts" of the 16-cell stage, for, though each of these four cells divides *twice more, all* the resulting cells are *prototrochal.*

The *secondary trochoblasts* (*Amphitrite* and *Clymenella*), on the contrary, contain other than prototrochal determinants. Each divides into two cells, of which one contains only *purely prototrochal* elements, while the other contains determinants of other structures also, and its division results in three prototrochal cells and one cell which becomes part of the general ectoderm.

When we trace back the history of the determinants of the primary and secondary trochoblasts respectively, we find that they are already separated in the 8-cell stage. At this stage the former are contained in the four upper, the latter in three of the lower cells. When these determinants control certain cells, even though the latter belong to different generations, they all unite to form one organ, the prototroch. However,

there is a good deal of symmetry in the formation of this organ, since each quadrant contributes exactly the same number of cells and in the same manner, except that the dorsal quadrant furnishes no secondary prototrochal cells.

On the other hand, in *Amphitrite* the determinants of the paratroch — a strictly bilateral organ — are all within one cell, the somatoblast, of the 16-cell stage, and at the next division the determinants of *three* of the four cells of the paratroch come to lie in one of the resulting cells, and those of the fourth in the other. Though the succeeding cleavages of these two somatoblast cells are quite different, nevertheless, after several divisions, the determinants come together again in control of four cells, which are bilaterally placed with respect to the larva and belong to the same generation, — the eleventh, counting the ovum as the first. That this is significant is indicated by the fact that two extremely minute cells are "budded off" just before the complete differentiation of the paratroch. Otherwise, of course, two of the cells would belong to the tenth generation.

When the origin of the prototroch and of the paratroch has been ascertained in a larger number of forms, we shall be in a position to discuss whether the destiny of cells is conditioned upon their contained determinants or upon their position.

III. Axial Relations.

The discussion of the axial relations of the egg of *Amphitrite* naturally falls into two chapters: the *orientation of the egg* with respect to the future sagittal plane, anterior and posterior end of the larva, etc.; and the *regional metamorphosis* or shifting of areas which occurs in consequence of the transformation of the one-layered blastula into the three-layered larva.

Nothing is known of the orientation of the egg until the first maturation spindle is formed; the direction of this indicates the future position of the polar globules and these in turn indicate the position of the anterior end of the trochophore, or the animal pole of the egg; 180° opposite lies the vegetative

pole. A line connecting the two poles is the egg axis. We can distinguish neither the prospective sagittal plane, nor the right and left sides of the trochophore, until the first cleavage spindle appears. This spindle indicates the direction and position of the first furrow, and all the other furrows follow in perfectly regular and constant sequence. At the 4-cell stage the prospective sagittal plane cuts *B* and *D* as in most annelids,— *Clepsine*, *Rhynchelmis*, *Clymenella*, *Aricia*, *Polymnia*, etc.; and in *Unio* and *Discocœlis*. The cells *A*, *B*, *C*, and *D* are the anlagen respectively of the left, ventral, right, and dorsal quadrants of the trochophore. Therefore, the second cleavage furrow does not coincide with the sagittal plane of the embryo.

When the four cells divide into eight, the upper quartette alternates with the lower so that the second cleavage furrow is twisted and its course more nearly coincides with the sagittal plane on the upper than on the lower hemisphere. From this time the number of cells increases in geometrical progression up to sixty-four, without effecting any considerable redistribution of material. The sixty-four cells are disposed in sixteen alternating quartettes, and the egg is capable of very precise orientation with respect to the sagittal plane, anterior and posterior ends, ventral and dorsal, right and left sides.

Now, if the second furrow is followed around the whole egg, its course is found to be an irregular zigzag, but its general direction is at a considerable angle to the sagittal plane. The embryonic material is segregated in such a way that the mesoderm is contained in one cell, the entoderm in seven; of the fifty-six remaining ectoderm cells, sixteen are already functioning as prototroch and four constitute the somatic plate. The prototrochal cells naturally divide the embryo into an umbrellar and a subumbrellar hemisphere. On the latter the principal shifting of areas, which characterizes the regional metamorphosis, takes place. The regions which figure as units during the transformation are the somatic plate, the mesoderm and entoderm, and the remaining subumbrellar ectoderm.

The main features of the metamorphosis are as follows: the mesoderm and entoderm sink into the segmentation cavity, and the somatic plate thins out and extends over the large area thus

vacated, besides still occupying its original territory. The manner in which this extension takes place is significant. — Except for a slight early movement of the plate backward, so that its edge reaches the vegetative pole in the middle line, all this extension is lateral and the material moves only in the arcs of small circles parallel to the prototroch or equator of the egg (p. 250). Thus, of course, the material just at the vegetative pole does not move. The lateral edges of the plate on either side come together and concresce in the mid-ventral line, and separate the blastopore or stomodæum from the posterior end.

As a result of the metamorphosis, the entoderm and mesoderm pursue their respective destinies inside the segmentation cavity, while the somatic plate occupies the greater part of the area of the subumbrella, including, of course, the posterior end, the entire dorsal and lateral region (excepting a small tract just behind the interruption of the prototroch, in the mid-dorsal line, occupied by cells from the umbrella), and a large portion of the ventral area: all the material lies practically in the same *latitude* as at first. At this point a comparison of *Amphitrite* with *Nereis* is of special interest: —

With regard to the axial relations, Wilson maintains two theses upon which he bases many far-reaching interpretations.

First, the second cleavage furrow coincides with the future sagittal plane of the embryo. Since one of the resulting cells is larger than the others, the embryo is one-sided in the early cleavage, and the cause of the transition from the spiral (oblique) to the bilateral cleavage is the reduction of this posterior macromere to the size of its fellow on the opposite side (Wilson,[1] p. 445). Now in *Amphitrite* and *Clymenella* the second furrow is inclined to the future sagittal plane so that the organism may be considered bilateral from the start, and the introduction of bilateral cleavage must be referred to an entirely different cause (cf. p. 241). And at no time does the second cleavage furrow, as a whole, even approximately separate the material of the right and left sides of the embryo. Since the cleavage of *Nereis* is essentially like that of these forms, the coincidence of the second cleavage furrow with the sagittal plane may well be questioned. The error seems to be in mis-

taking a small part of the furrow, that between the entomeres, for the whole (cf. Lillie, p. 42).

Second, during the regional metamorphosis, the "ventral plate" shifts through an angle of about 90°, so that the main portion occupies the ventral region of the trunk, and the teloblastic area, which lay in the dorsal region near the prototroch, comes to lie at the posterior pole. This leaves the latero-dorsal and mid-dorsal areas to be accounted for in another way.

From this thesis Wilson deduces his theory of the shifting of the neural axis, and of the homology of the posterior teloblasts with the neuro-nephroblasts of the Oligochætes and Hirudinea, and elaborates a scheme for harmonizing the apparently irreconcilable metamorphosis of various larval forms,—*Polygordius, Clepsine, Lumbricus, Lopadorhynchus*, etc. (Wilson,[1] p. 426). The phenomena of axial metamorphosis in *Amphitrite* are utterly at variance with the terms of this second thesis, and therefore do not substantiate the interpretations which rest upon them (cf. p. 250, text Fig. IV).

To return to *Amphitrite*. During the regional changes on the lower hemisphere, the four paratrochal cells, which at first lay in a row at the posterior lip of the blastopore, are brought round to form a circle near the posterior end of the larva, parallel with the prototroch; and the cells of the somatic plate, concrescing in the mid-ventral line, separate the paratroch from the stomodæum. From this time the trochophore elongates in the direction of the original egg axis, by the division of the cells of the budding zone, which lie anterior to the prototroch; the latter persists until the trunk develops several metameres, and always belongs to the ultimate segment of the body.

Few changes take place upon the anterior hemisphere which affect the axial relationships of the larva. The dorsal umbrella mucous glands, whose cytogenetic origin is known, may be relied upon as orienting points until the larva has several setigerous metameres; and the median tentacle, since it bears a constant relation to the mucous glands, orients this region after their disappearance.

University of Chicago,
May, 1895.

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REFERENCE LETTERS.

The quadrants of the egg are indicated by the letters *a*, *b*, *c*, and *d*. The cells at the two poles of the eggs are distinguished by these letters with figures as subscripts, *e.g.*, A_3, B_4, etc., at the vegetative pole, $a_{1.2}$, $b_{1.2}$, etc., at the animal pole; for the other cells the figures are written as exponents, *e.g.*, $a^{2.1}$, $b^{3.2}$. In general, the upper product of a cell division receives the smaller exponent, thus:

$$a^2 \begin{cases} a^{2.1} \begin{cases} a^{2.1.1} \\ a^{2.1.2} \end{cases} \\ a^{2.2} \begin{cases} a^{2.2.1} \\ a^{2.2.2}\ * \end{cases} \end{cases}$$

The following abbreviations are also used: †

*,	landmark, first seen in Fig. 35.
bp.,	blastopore.
ent.,	entoderm.
gl.l.,	left dorsal umbrellar mucous gland.
gl.rt.,	right " " " "
gl.,	other umbrellar mucous glands.
l^1, l^2, l^3,	cf. reference to Fig. 34.
mem.,	egg membrane.
M,	mesoderm proteloblast.
m m,	anterior products of first division of paired mesoblasts.
M M,	paired mesoderm cells, or teloblasts of mesoderm bands.
mes.,	mesoderm bands.
par.,	paratroch.
par.v.lft.,	left ventral paratrochal cell.
par.v.rt.,	right " " "
par.d.lft.,	left dorsal " "
par.d.rt.,	right " " "
proc.,	proctodæum, or anlage of same.
p.g.,	polar globules.
prot.,	prototroch.
som.pl.,	somatic plate.
stomod.,	stomodæum.

* The nomenclature is that of Wilson[1] somewhat modified, p. 230.

The blue tint is used to distinguish the "cross" and the somatic plate; brown, the prototroch; light red, the mesoderm; the entoderm is stippled.

† Other abbreviations used but once are explained in the special reference to the figure. For substitution letters, ap^1, ap^4, etc., see table, p. 236.

DESCRIPTION OF PLATES.

All the figures have been drawn with the aid of the camera lucida. No attempt has been made in most cases to represent the cell structure; on the contrary, various schematic devices have been introduced, *i.e.*, colors, heavy or dotted lines, shaded nuclei, etc., to assist one in following the more important groups of cells, although they certainly detract from the faithfulness of the representations. I have endeavored to select figures which represent every cell division up to a late stage in *Amphitrite*. In the very late stages and in the figures of the eggs of other species I have, for reasons of economy, left out many intermediate drawings and indicated the origin of cells by arrows only. The size of the figures has been determined by convenience and does not represent the relative size of the eggs.

EXPLANATION OF PLATE X.

Amphitrite ornata Verrill.

FIG. 1. Unsegmented egg, viewed from animal pole, showing first cleavage spindle inclined to future sagittal plane of embryo (line joining *x* and *y*), and nearer one end of the egg; *l* and *r*, left and right; *x*, ventral, *y*, dorsal; *m*, egg membrane.

FIG. 2. The 2-cell stage, viewed from vegetative pole: *AB* and *CD*, first two blastomeres.

FIG. 3. Viewed from one end: shows the spindle of first two blastomeres inclined to meridian at an early period; *p.g.*, polar globules; *an.*, animal pole; *veg.*, vegetative pole.

FIG. 4. Division of first two blastomeres nearly completed, viewed from vegetative pole: *A*, *B*, *C*, and *D*, first four blastomeres, the anlagen, respectively, of the left, ventral, right, and dorsal quadrants.

FIG. 5. The 4-cell stage, viewed from animal pole: karyokinetic figures not all of same age; *I–I*, first cleavage furrow; *II–II*, second cleavage furrow.

FIG. 6. Side view: division of the four cells into eight.

FIG. 7. The 8-cell stage from below (vegetative pole): *a*, *b*, *c*, and *d*, the four upper cells which constitute the umbrellar or upper hemisphere; A_1, B_1, C_1, and D_1, four lower cells which constitute the subumbrellar or lower hemisphere; *cr.f.*, cross-furrow; *seg.cav.*, segmentation cavity.

FIG. 8. From animal pole: transition from 8-cell to 16-cell stage. The lowermost products of the division of the four uppermost cells are the primary trochoblasts $a^{1.1}$, $b^{1.1}$, $c^{1.1}$, and $d^{1.1}$.

FIG. 9. From lower pole: transition from 8 to 16-cell stage.

FIG. 10. From left side: transition from 8 to 16-cell stage.

FIG. 11. 16-cell stage from above: nearly all the cells contain karyokinetic spindles in early stages which indicate the direction of the next cleavage.

FIG. 12. Same egg as Fig. 11 from right side: *seg.cav.*, segmentation cavity.

FIG. 13. Same egg as Figs. 11 and 22 from left side: the spindles in $b_{1.1}$, $c^{1.1}$, d^2, and D_2 are seen from the end; the spindle in $d^{1.1}$ is in an abnormal position; it is in all other eggs I have examined parallel with that in $d_{1.1}$.

FIG. 14. Same egg from vegetative pole.

FIG. 15. From vegetative pole: later than last figure; transition to 32-cell stage.

FIG. 16. 32-cell stage from animal pole: the furrows drawn with heavy lines, *II–II*, indicate the direction of the second cleavage furrow; those in dotted lines, *I–I*, that of the first cleavage furrow.

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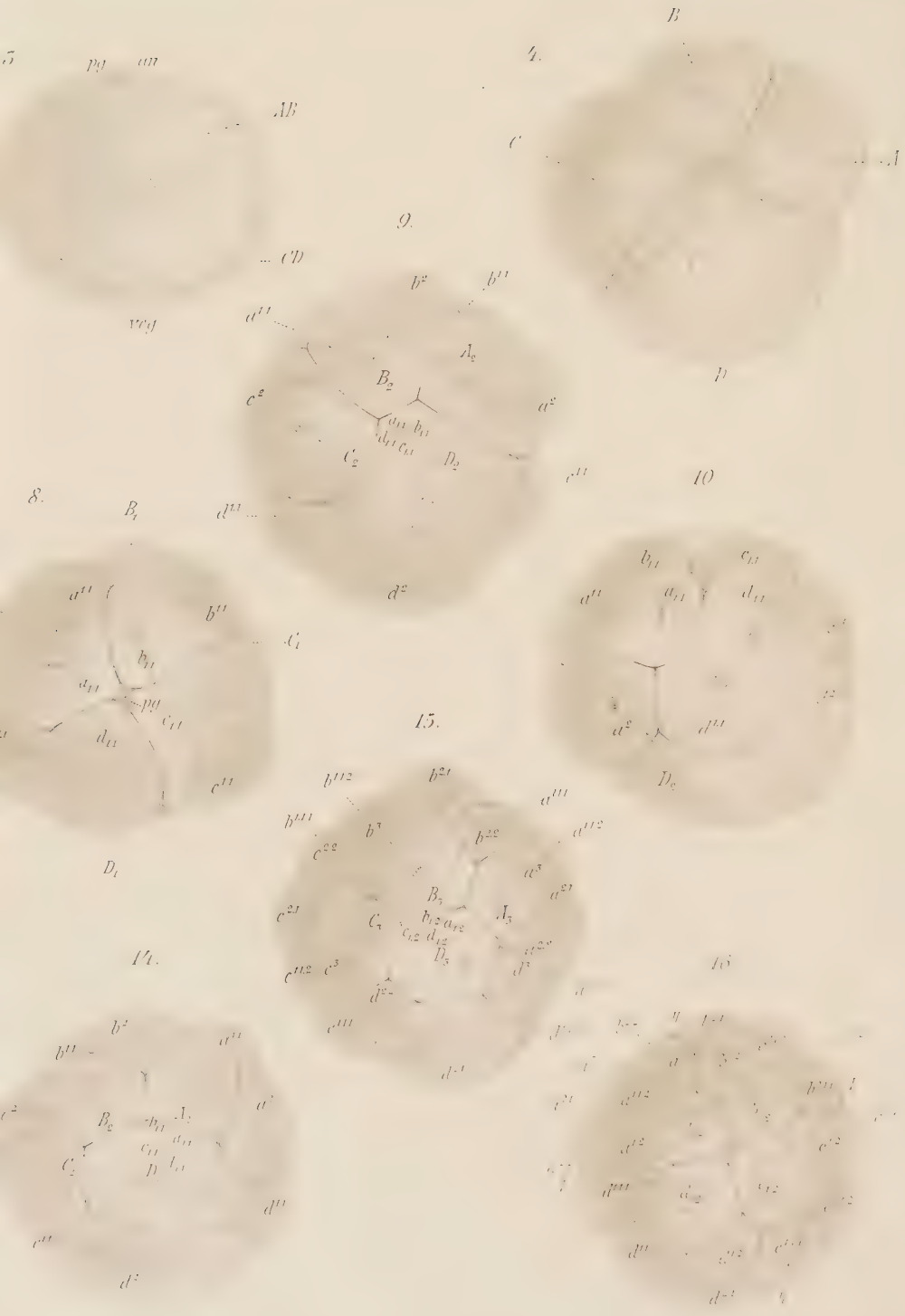

Lith. Anst. v. Werner & Winter, Frankfurt a/M.

EXPLANATION OF PLATE XI.

Amphitrite.

Cross and somatic plate, blue; prototroch, brown; mesoderm, red; entoderm, stippled.

FIG. 17. From animal pole: the spindles in the four apical cells indicate the divisions resulting in the rosette; end view of spindles in the primary trochoblasts of second generation (colored brown).

FIG. 18. From animal pole: the primary trochoblasts of third generation, *i.e.*, the cells of the primary prototroch (brown), have developed cilia; apical rosette, $a_{1\cdot3}$–$d_{1\cdot3}$; parent cells of cross in blue; trochophore now rotates in direction indicated by arrow, *ar*.

FIG. 19. From vegetative pole, about same age as Fig. 18: primary mesoblast cell *M*, or d^4, being formed (colored red); somatic-plate cells in blue; primary prototroch in brown as before.

FIG. 20. Same view: mesoderm cell fully formed; superficial position of *D*. The two figures show an unusual time variation, the division producing d^4 (= *M*) being tardy in Fig. 19; other cells colored as before.

FIG. 21. Same egg as Fig. 20 from right side: color as in previous figures; cross and somatic plate, blue, etc.

FIG. 22. Same egg from left side.

FIG. 23. From vegetative pole, somewhat later: entoderm plate stippled; only posterior prototrochal cells visible; ciliated.

FIG. 24. Like Fig. 18, somewhat later stage.

FIG. 25. From left and below: same egg as Fig. 23.

FIG. 26. From right side and below: same egg as Figs. 23 and 25.

FIG. 27. From left side, about same stage as last figure: shows ciliation of ap^1 (= $a^{1.1.1.1}$), which in *Nereis* is non-ciliated (Wilson).

FIG. 28. Same egg as Fig. 27 from ventral side.

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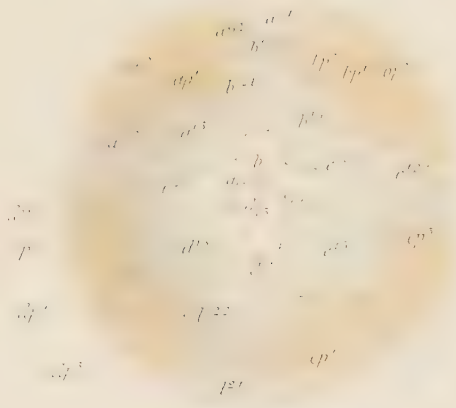

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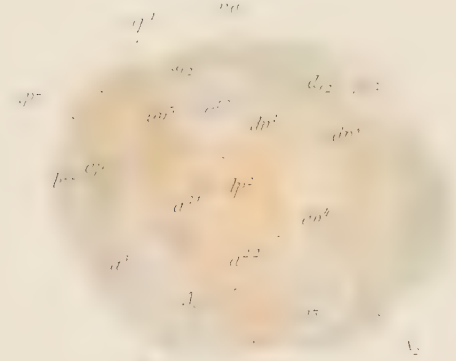

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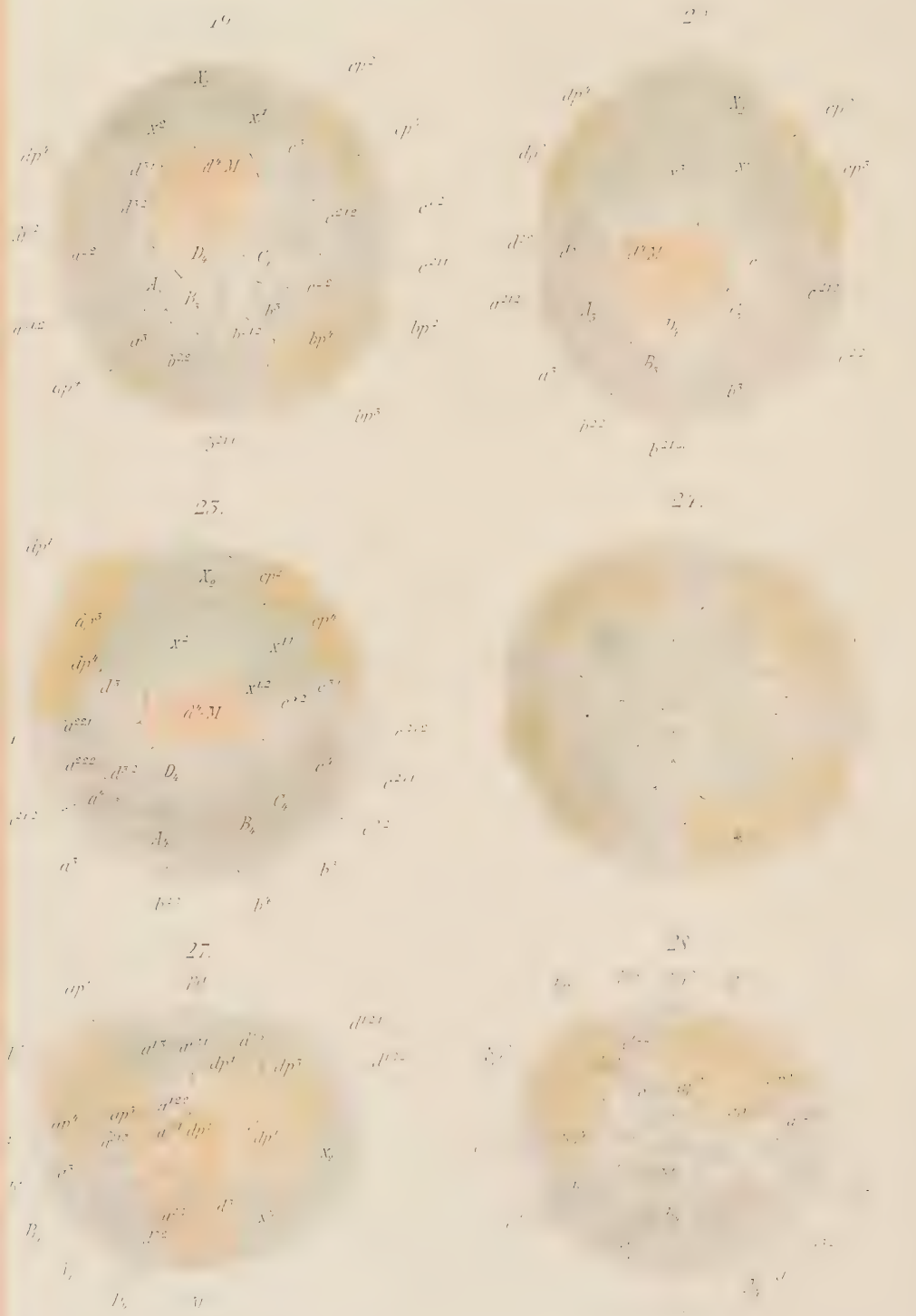
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EXPLANATION OF PLATE XII.

Amphitrite.

FIG. 29. From animal (apical) pole: cross, blue; rosette in middle; mesoderm cells (red) and somatic-plate cells show through.

FIG. 30. From animal pole, somewhat later: rosette cells dividing; $d^{1.2.2}$ divided, its lower products indicated by shaded nucleus; mesoderm and somatic-plate cells as before.

FIG. 31. Same view: further divisions of cross cells; rosette left out.

FIG. 32. Same view: rosette divided; *gl.l.* and *gl.r.*, left and right-dorsal mucous glands of umbrella, corresponding to "head-kidneys" in *Nereis* (Wilson); *g* and *g*, corresponding cells in ventral arms of cross; $d^{1.2.2.2}$ about to divide (cf. previous Figs. 31, 32); *gl.l.*, *gl.r.*, mucous glands.

FIG. 33. Same view: further divisions of the cross cells (rosette not figured), *g* and *g* dividing; compare Figs. 31, 32 *gl.l.*, *gl.r.* mucous glands.

FIG. 34. Same view: dorsal part of umbrella; the lower daughter cells of $d^{1.2.2.2}$ (Fig. 32) dividing; the three products are l^1, l^2, and l^3; *gl.l.*, *gl.r.*, mucous glands.

FIG. 35. From animal pole, somewhat dorsal: $d^{1.2.2}$ dividing to form the important landmark (*); $d^{1.2.1}$ divided; x^3 dividing.

FIG. 36. Nearly same view as Fig. 35, later stage: *gl.l.*, *gl.r.*, mucous glands.

FIG. 37. Same view, somewhat later, showing left mucous gland cell, *gl.l.*, nearly covered by surrounding cells.

FIG. 38. Lower hemisphere, about the same stage as Fig. 29: somatic plate, blue; mesoderm *M* and *M*, red; entoderm, stippled.

FIG. 39. Dorsal view, little later than Fig. 38.

FIG. 40. Lower (subumbrellar) hemisphere: shows secondary trochoblasts cpc^5, p^6, cp^7; ap^5, ap^6, ap^7; bp^5, bp^6, bp^7.

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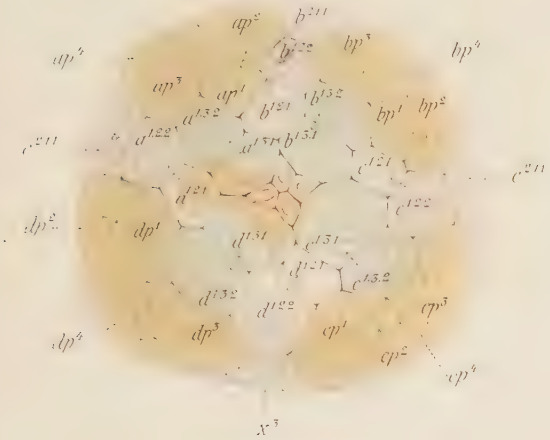

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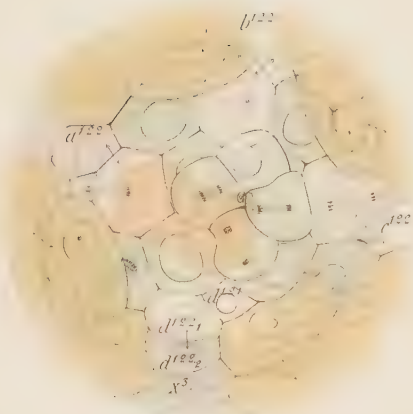

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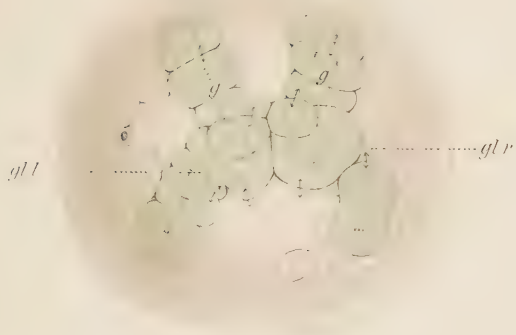

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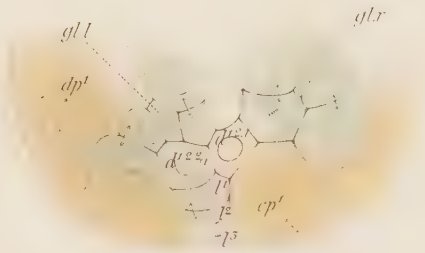

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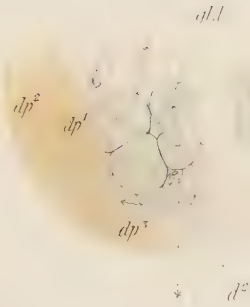

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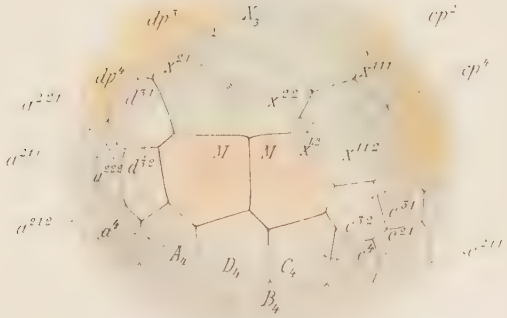

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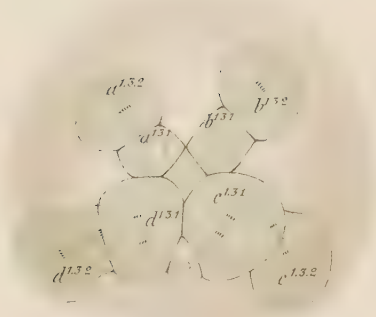

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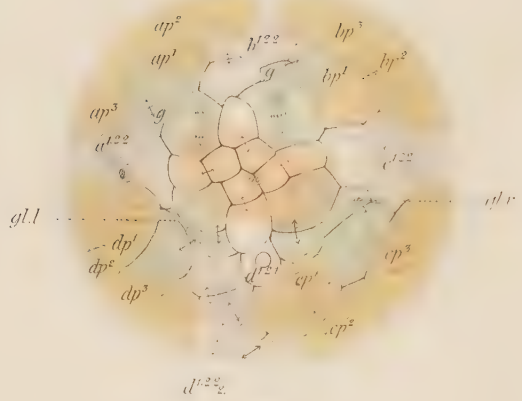

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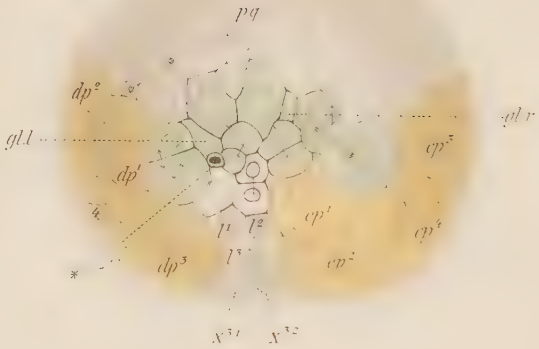

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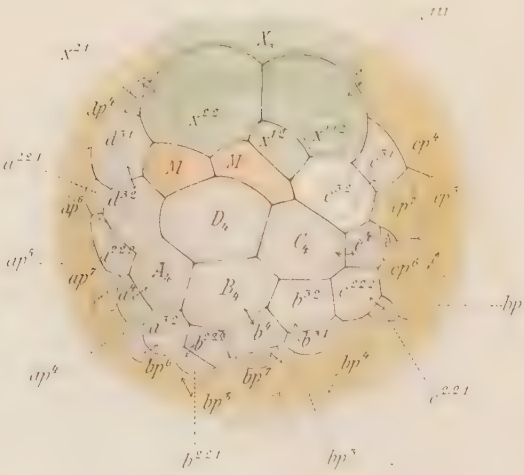

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EXPLANATION OF PLATE XIII.

Amphitrite.

Prototroch, brown ; somatic plate, blue ; mesoderm, red ; entoderm, stippled ; the groups of somatic-plate cells descended from X_3, x^2, and x^1 separated by heavy lines.

FIG. 41. Lower hemisphere : mesoderm cells beginning to sink below the surface.

FIG. 42. Same view : mesoderm cells *M* and *M* partly covered and ready to divide and give rise to the minute cells seen in Fig. 46, *m* and *m*.

FIG. 43. Similar view, somewhat to left side.

FIG. 44. Same view as last figure and about the same age. The two figures show the variations in *time* of division.

FIG. 45. Dorsal view, showing the dorsal interruption in the prototroch.

FIG. 46. Lower hemisphere, dorsal : cells of entoderm plate dividing for the last time on the surface ; ends of mesoderm cells still seen at the surface ; *m* and *m*, small cells in segmentation cavity arising by division of the original mesoderm cells *M* and *M* (cf. Figs. 42 and 44).

FIG. 47. Lower hemisphere : definitive entoderm plate ; mesoderm entirely within the segmentation cavity.

FIG. 48. Dorsal view : division of x^3.

FIG. 49. Lower hemisphere, dorsal.

FIG. 50. Lower hemisphere, dorsal : mesoderm cells below the surface ; the mesodermal teloblasts ready to divide the second time.

FIG. 51. Dorsal view, showing interruption of prototroch.

FIG. 52. Lower hemisphere : each mesoblast band composed of three cells.

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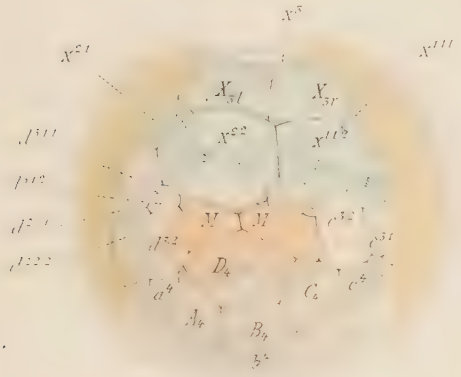

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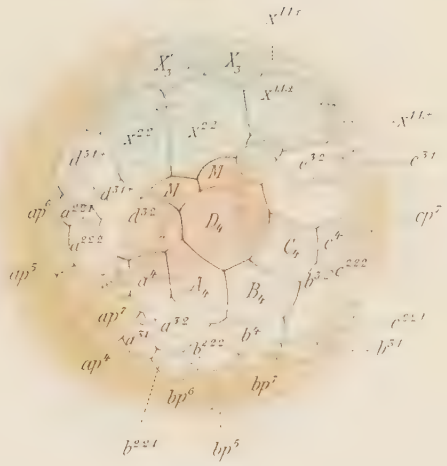

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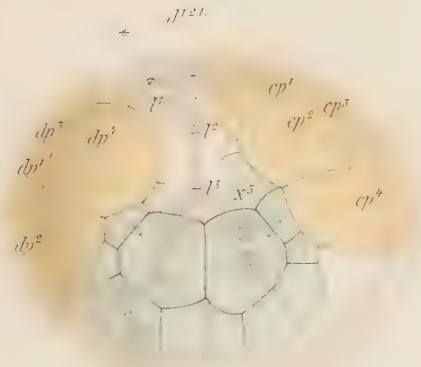

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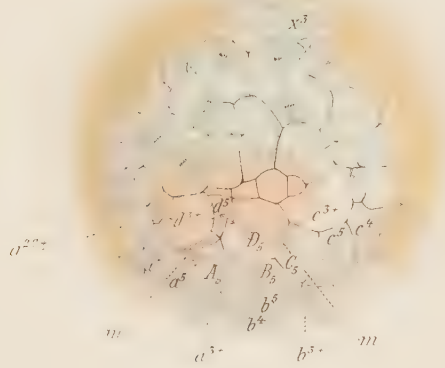

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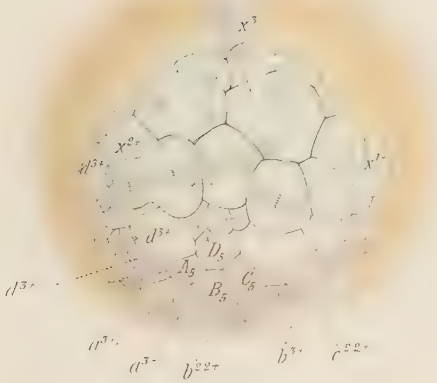

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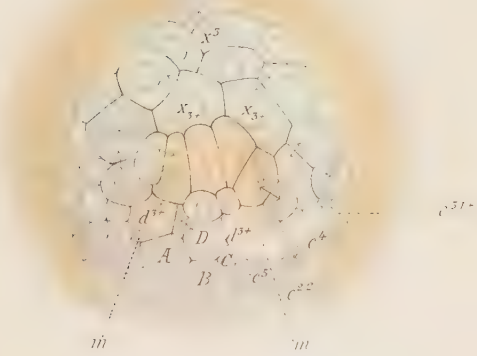

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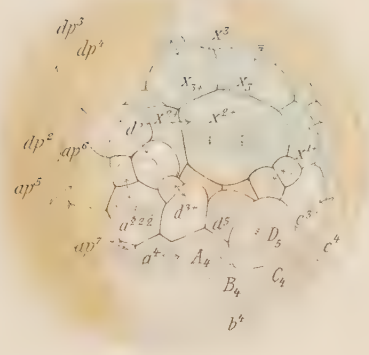

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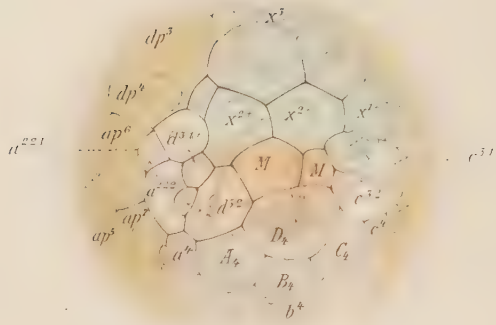

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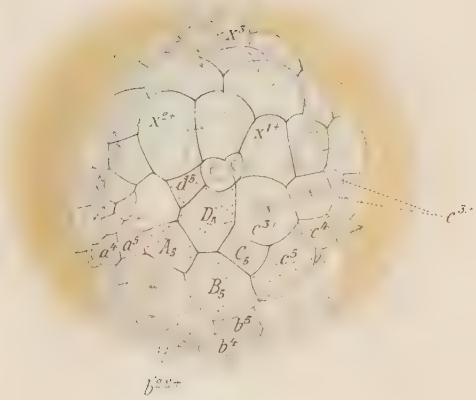

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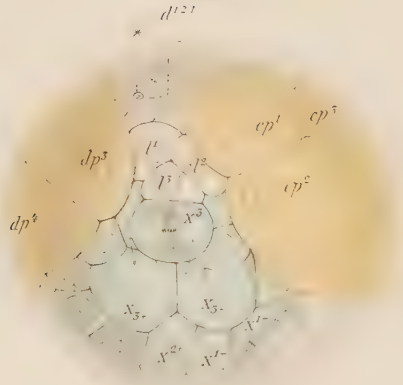

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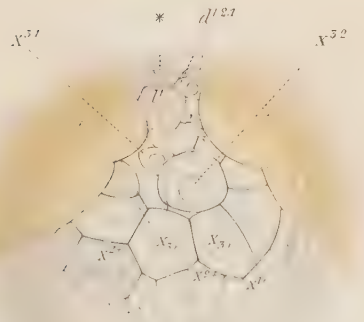

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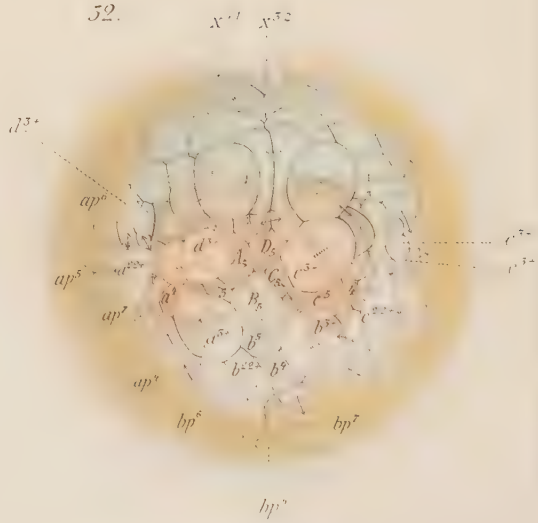

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EXPLANATION OF PLATE XIV.

Amphitrite.

Prototroch and *paratroch* brown; somatic plate, blue; mesoderm, red; entoderm, stippled.

FIG. 53. Lower hemisphere, dorsal: *par.v.lft.*, left-ventral paratrochal cell; *par.v.rt.*, right-ventral paratrochal cell; the two cells between them give rise after one division (cf. next figure) to the dorsal paratrochal cells.

FIG. 54. Same view: last division of paratrochal cells; *par.d.lft.*, left-*dorsal* paratrochal cell; *par.v.lft.* and *par.v.rt.*, left and right-ventral paratrochal cells.

FIG. 55. Same view, later: paratroch completely differentiated; *par.d.rt.*, right-*dorsal* paratrochal cell; others as in Fig. 54.

FIG. 56. Lower hemisphere: beginning of concrescence of somatic-plate cells from either side ventral to the paratroch; *proc.*, area of future proctodæum.

FIG. 57. Same view, later: further concrescence of somatic plate: letters x^2, $d^{3.1}$, etc., indicate from what cells the respective areas are derived.

FIG. 58. Dorsal view of stage like Fig. 56: interruption of prototroch; cells with shaded nuclei; 1^1, 1^2, 1^3, etc., descended from $d^{1.2.2}$ (cf. Fig. 30).

FIG. 59. Dorsal view: *gl.* and *gl.*, dorsal umbrellar mucous glands (cf. Figs. 32, 33, etc.); mesoderm bands, *mes.*, composed of six cells each; interruption in prototroch nearly completed; other letters as before.

FIG. 60. Dorsal view: prototroch interruption obliterated by concrescence of prototroch cells.

FIG. 61. Right side: surface view of prototroch; the rest in optical section, showing entoderm cells, *ent.*, somatic plate, *som.pl.*; mesoderm band — three cells, teloblast dividing, *mes.*; paratroch cells, *par.d.rt.*, *par.v.rt.*, etc.

FIG. 62. Left side: same egg as Fig. 59; entoderm and mesoderm bands in the interior; letters as before.

FIG. 63. Right side, later larva, showing prototroch, paratroch, stomodæum (*stomod.*), etc.

FIG. 64. Optical section from left side: *prob.*, problematic bodies; *gl.l.*, left-dorsal umbrellar mucous gland (cf. Figs. 30 and 59); *gl.*, ventral umbrellar mucous glands; *duct*, duct of gland; *ext.op.*, opening of duct to exterior; *vac.*, vacuoles in cells of prototroch; *stomod.*, stomodæum; *muc.*, mucous glands of subumbrella; *n*, groove separating head segment from first trunk segment; *par.v.lft.* and *par.d.lft.*, ventral and dorsal paratroch cells of the left side; *proc.*, proctodæum. For later stages see text Figs. XI–XVIII.

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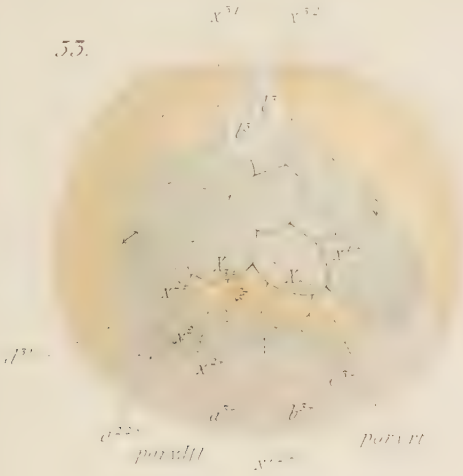

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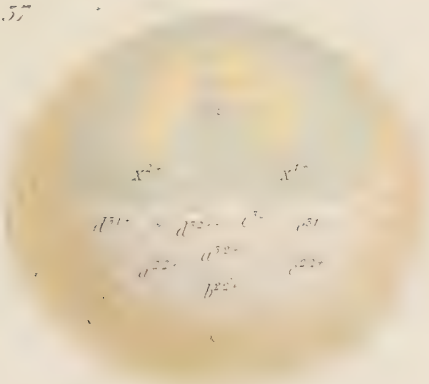

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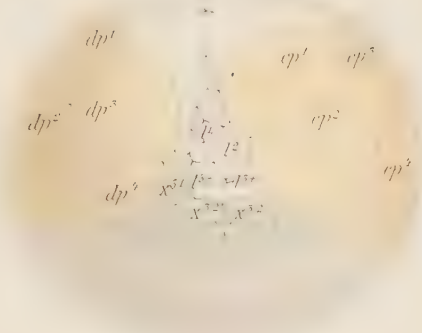

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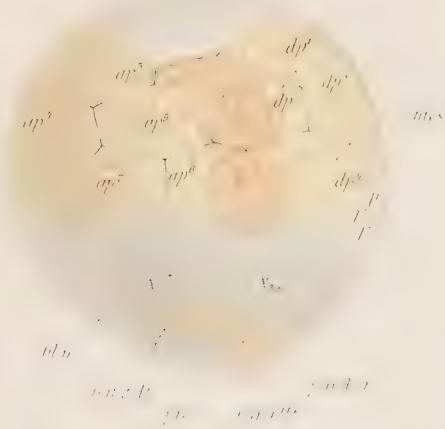

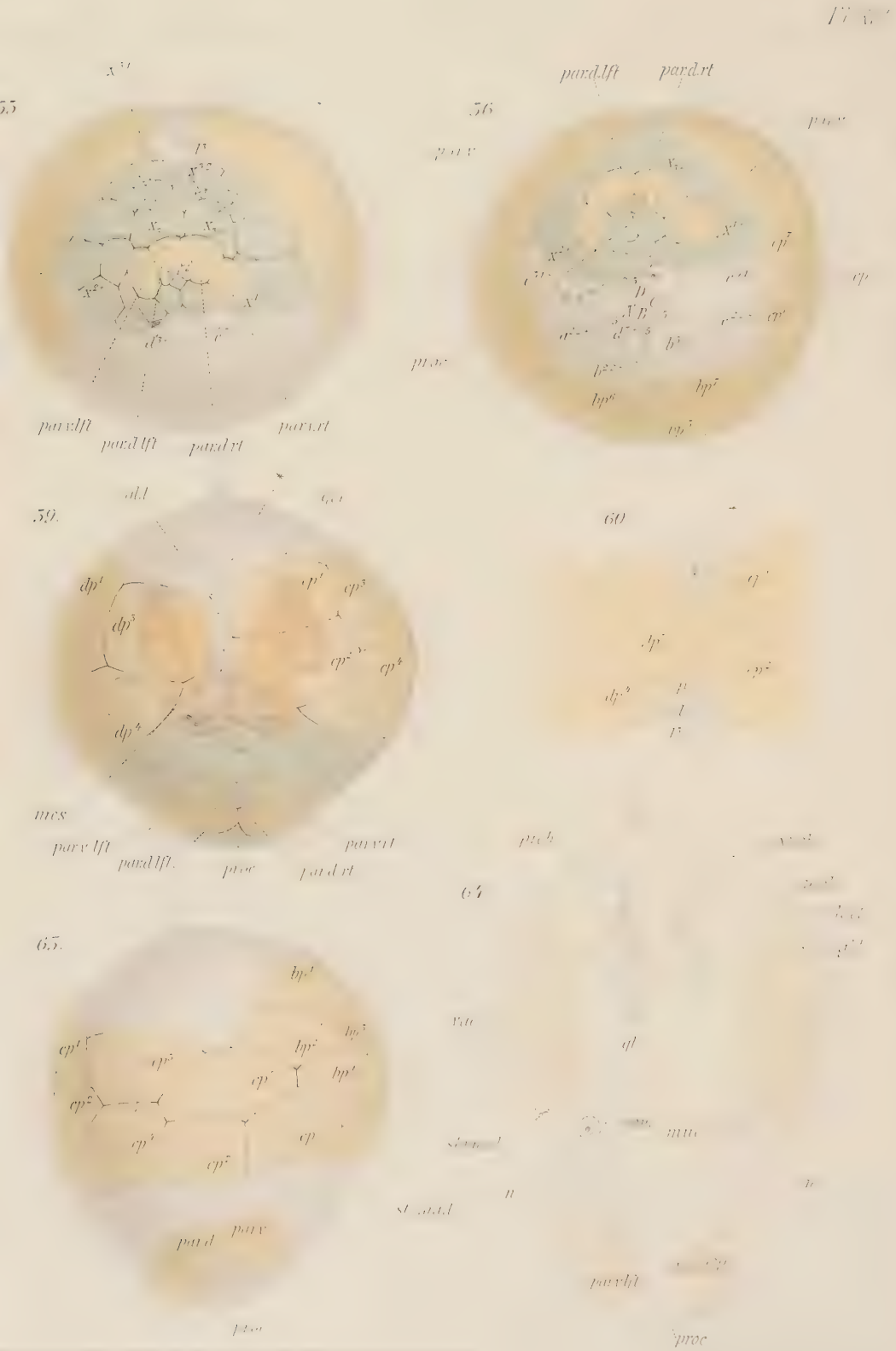

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EXPLANATION OF PLATE XV.

Clymenella torquata Verrill.

FIG. 65. From animal pole (nearly) : 2-cell stage.

FIG. 66. Same view : 4-cell stage.

FIG. 67. From vegetative pole : 8-cell stage.

FIG. 68. 16-cell stage from animal pole : from life.

FIG. 69. Same view : thirty-two to sixty-four cells ; formation of "apical rosette."

FIG. 70. Same view : primary prototroch complete

FIG. 71. Same view : rosette cells dividing.

FIG. 72. Same view : two of the rosette cells have divided ; *gl.l.* and *gl.rt.* correspond to mucous gland cells in *Amphitrite*, "head-kidneys" in *Nereis*

FIG. 73. Lower hemisphere : 32-cell stage ; from life.

FIG. 74. Same view : 32 to 64-cell stage ; formation of mesoderm cell $M = d^4$.

FIG. 75. Right side : same stage.

FIG. 76. Left side : about the same stage.

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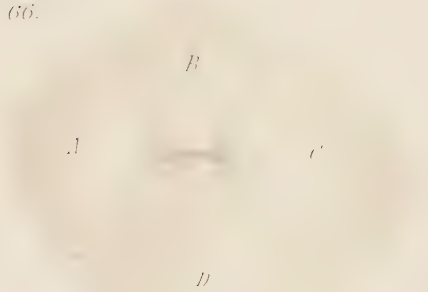

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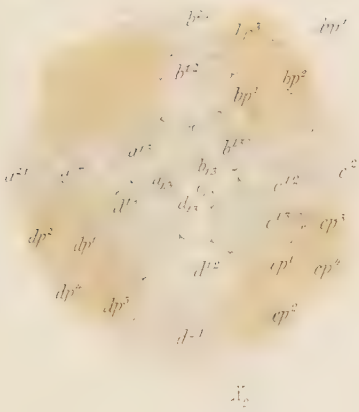

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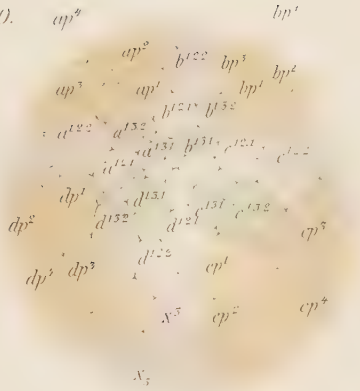

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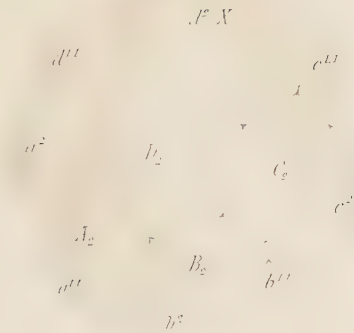

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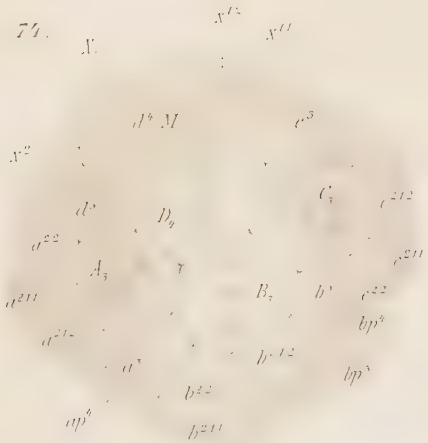

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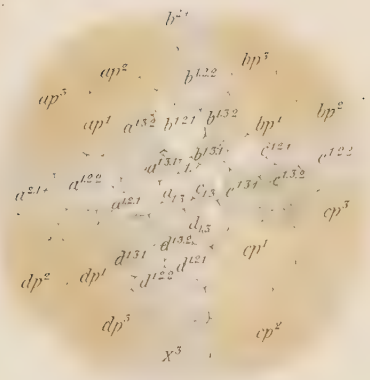

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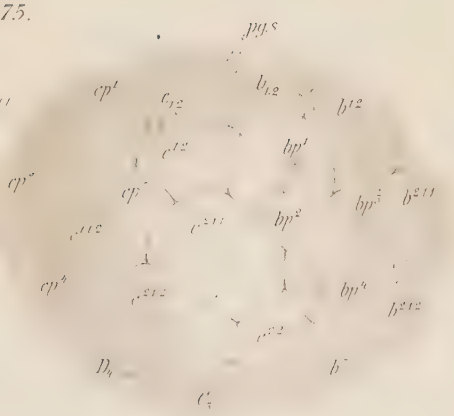

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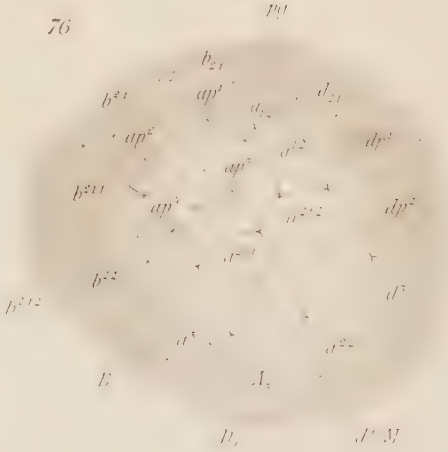

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EXPLANATION OF PLATE XVI.

Clymenella.

FIG. 77. Left side: later than last figures; first division of secondary trochoblasts.

FIG. 78. Lower hemisphere: about the 64-cell stage.

FIG. 79. Dorsal view: about the same stage.

FIG. 80. Left side: after 64-cell stage; formation of secondary prototrochal cells ap^5, ap^6, ap^7.

FIG. 81. Right side: same egg as Fig. 77.

FIG. 82. Lower hemisphere: same stage; mesoderm ready to divide.

FIG. 83. Same view, a little later.

FIG. 84. Lower hemisphere, later: last surface division of entoderm-plate cells — mesoderm divided.

FIG. 85. Same view, later.

FIG. 86. Same view: mesoderm cells getting ready to divide again; vacuoles in three entoderm cells, A_5, B_5, and C_5, indicated by faint lines; also the outlines of X_3 and X_3; entoderm plate complete; prototroch complete.

FIG. 87. Same view, little later stage.

FIG. 88. Lower hemisphere, somewhat dorsal: mesoderm cell divided; *m* and *m* correspond to the very minute cells in *Amphitrite* (cf. Fig. 50, *m* and *m*).

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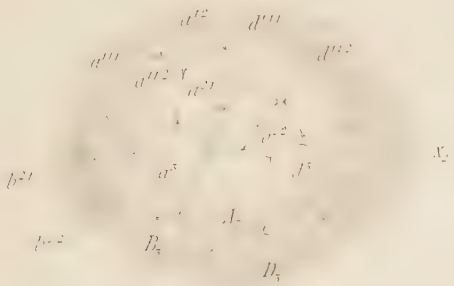

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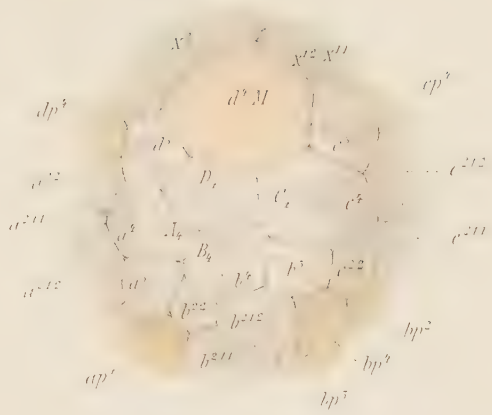

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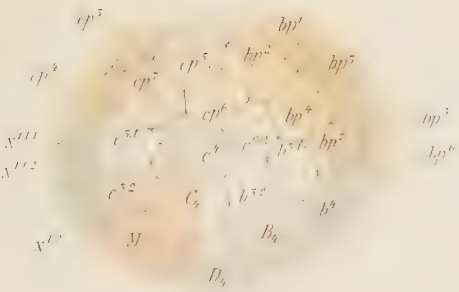

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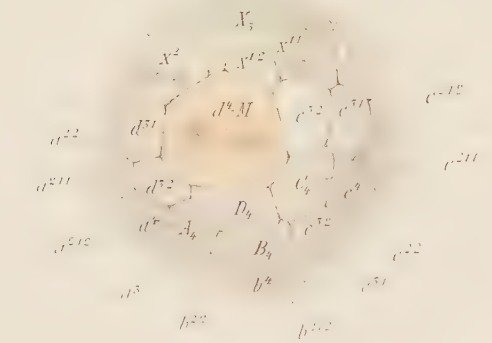

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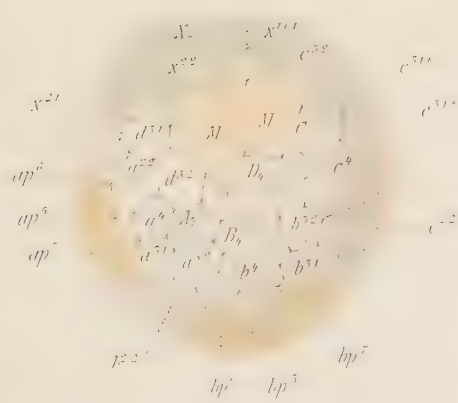

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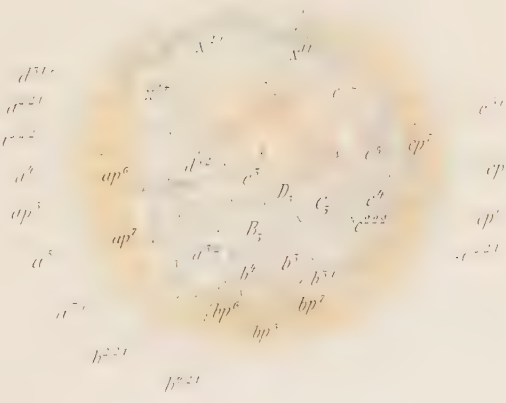

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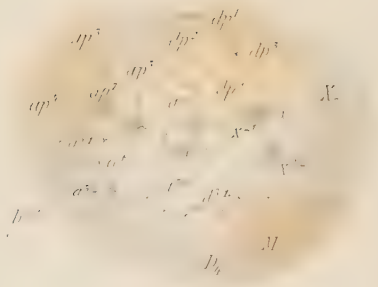

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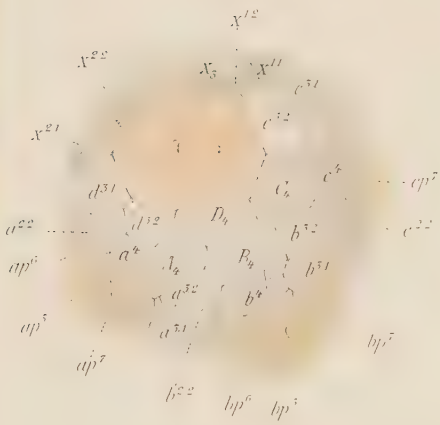

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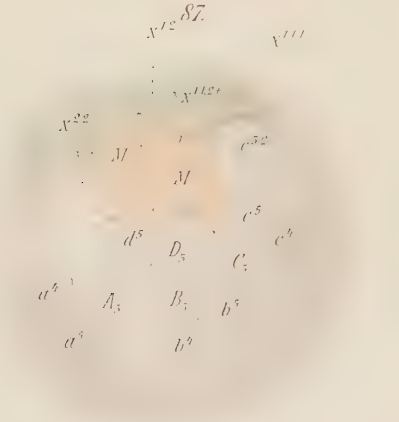

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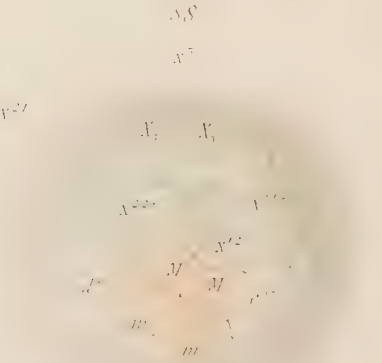

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EXPLANATION OF PLATE XVII.

Lepidonotus sp.

FIG. 89. Side view, showing that the first furrow sinks in at the animal and vegetative pole at the same time; *mem.*, egg membrane; solid line 1, outline at 11.27 P.M.; 2, outline of furrow at 11.28; 3, at 11.29; 4, at 11.31 P.M.; *p.g.*, polar globules.

FIG. 90. From animal pole: transition from 4 to 8-cell stage; cross-furrows at right angles to each other.

FIG. 91. From animal pole: 8-cell stage from life.

FIG. 92. Side view: 16 to 32-cell stage (cf. *Amphitrite*, Figs. 12 and 21, Pls. X, XI).

FIG. 93. From animal pole: thirty-two to sixty-four cells (cf. *Amphitrite*, Fig. 17); *pg.*, polar globules *inside* the apical cells.

FIG. 94. Lower (vegetative) hemisphere of same egg.

FIG. 95. Similar stage from vegetative pole: transparent; the cross and rosette cells (animal pole) in dotted lines.

FIG. 96. Similar stage from side, showing segmentation cavity.

FIG. 97. Side view, later: trochophore begins to swim; shaded cells are prototrochal (cf. Figs. 27, 28, *Amphitrite*, 77 and 80, *Clymenella*).

FIG. 98. Actual section of stage like Fig. 96 shows polar globules inside of apical cells, *p.g.*; segmentation cavity, *seg.cav.*

FIG. 99. Same egg as Fig. 96, from animal pole. Arrow indicates direction in which the trochophore rotates.

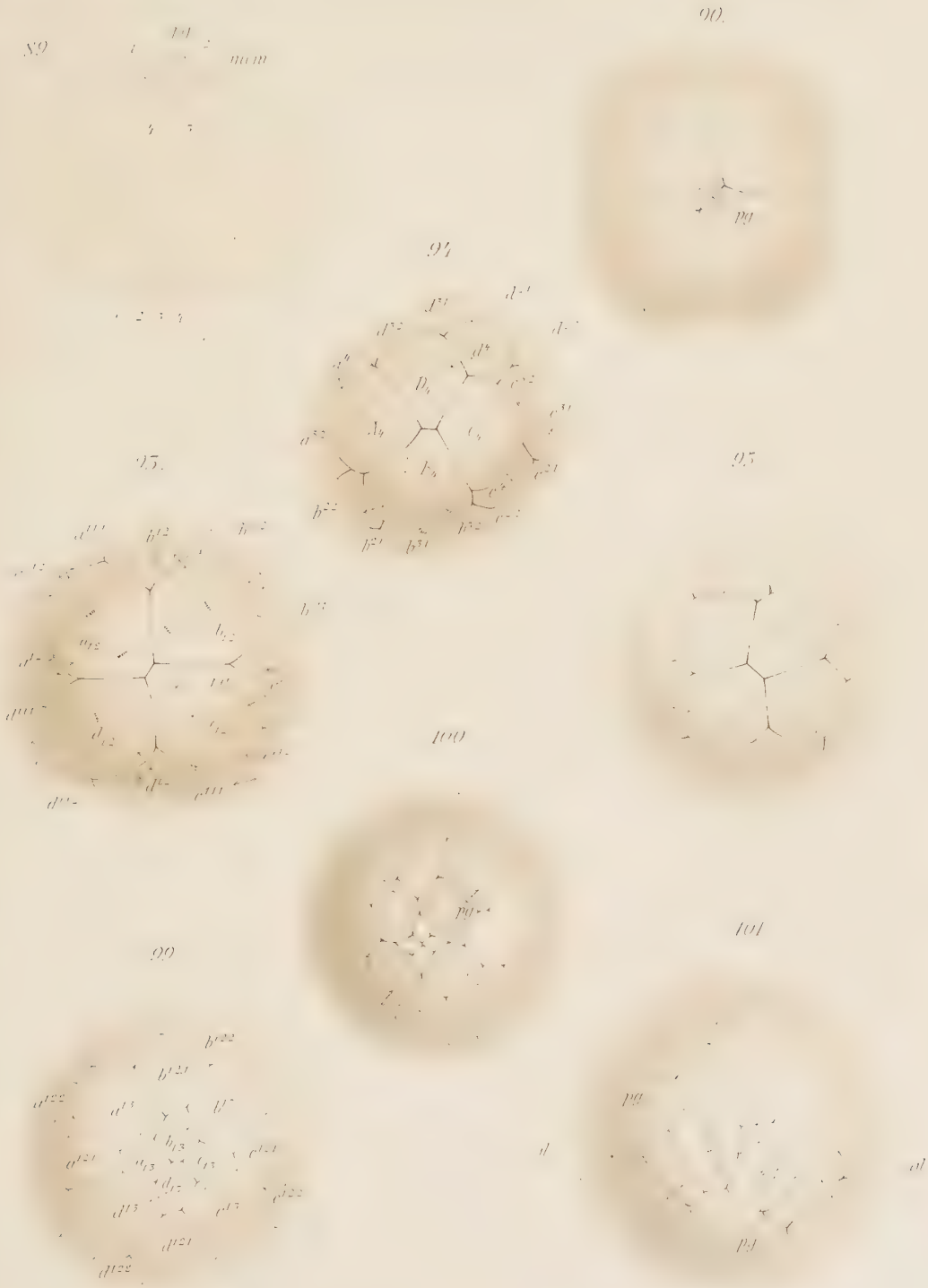
FIG. 100. Later stage: cross shaded, two cells in each arm (cf. Fig. 29); *p.g*, one polar globule, — the other within segmentation cavity.

FIG. 101. From animal pole: rosette and part of cross; three cells in some of the arms of cross, probably in all; *gl.*, cell corresponding to mucous gland in *Amphitrite* (cf. Fig. 32).

FIG. 102. Optical vertical section: the nuclei of the eight elongated vegetative cells are migrating inward. The cells 4, 4, are a^4, b^4, c^4, or d^4.

FIG. 103. Actual section, vertical, somewhat later.

FIG. 104. Optical section, gastrula: *p.g.*, polar globule; *prot.*, prototroch; *mes.*, mesoderm cell probably; *bp.*, blastopore.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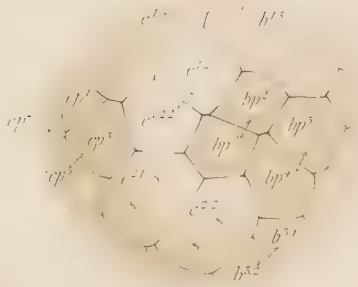

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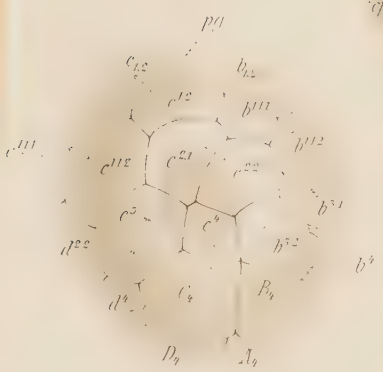

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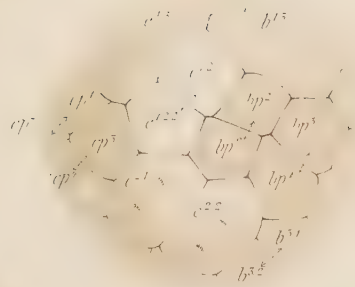

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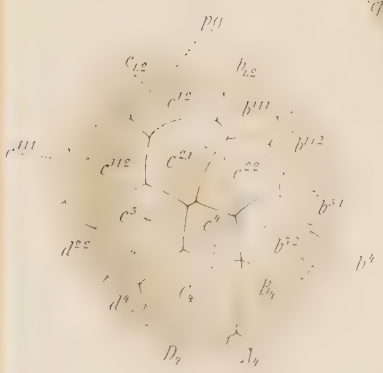

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EXPLANATION OF PLATE XVIII.

Scolecolepis viridis Verrill.

FIG. 105. From life, side view: first polar globule, *p.g.*; *m*, egg membrane.

FIG. 106. From left: 2-cell stage, vegetative pole. Precocious formation of cross-furrow.

FIG. 107. From life: three cells, seen from animal pole.

FIG. 108. From life: 4-cell stage, seen from animal pole.

FIG. 109. From life: animal pole and side.

FIG. 110. From life: 16-cell stage.

FIG. 111. Vegetative pole: same stage.

FIG. 112. 16-cell stage: cells tinted brown correspond to the *primary trochoblasts* of *Amphitrite* and *Clymenella*.

FIG. 113. Lower hemisphere, after division of cells seen in Fig. 110: from life.

FIG. 114. From animal pole: cells tinted brown as before; cells $a^{2.1}$, $b^{2.1}$, and $c^{2.1}$ correspond to the *secondary trochoblasts* of *Amphitrite* and *Clymenella*.

FIG. 115. Trochophore with three trunk segments; *eye*, eye-spot; *prot.*, prototroch; *par.*, paratroch; *m.*, mouth; *I*, *II*, *III*, trunk segments.

FIG. 116. Optical section, side: *muc.*, mucous gland; *prot.*, prototrochal cells; *ent.*, entoderm; *mes.*, mesoderm.

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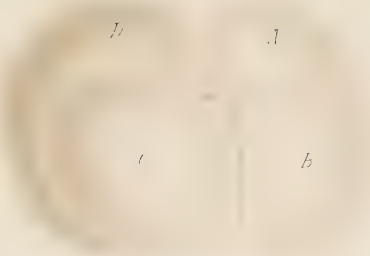

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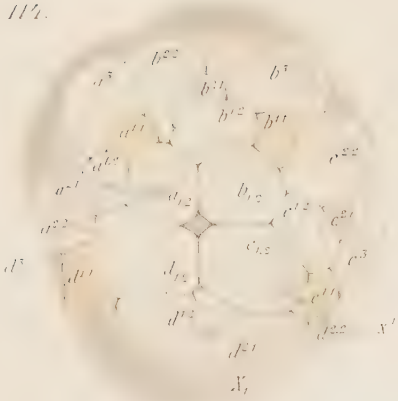

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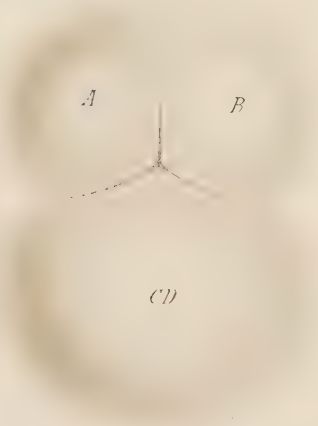

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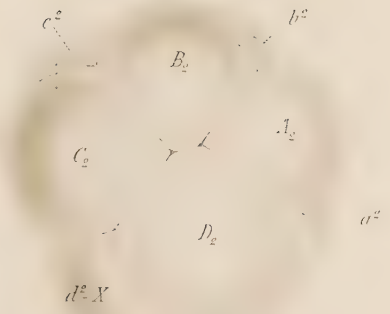

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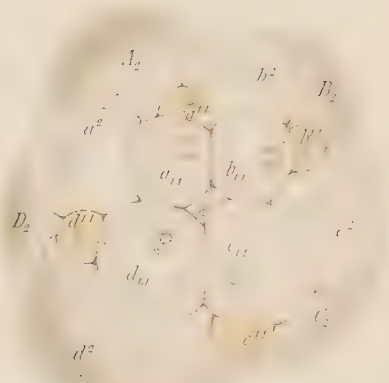

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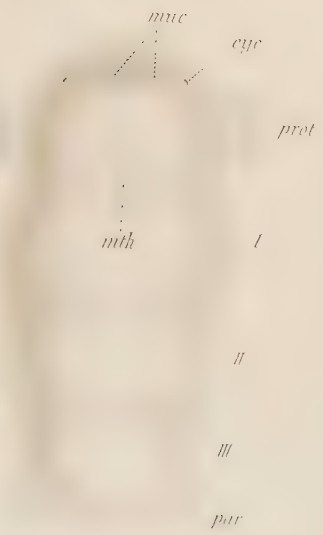

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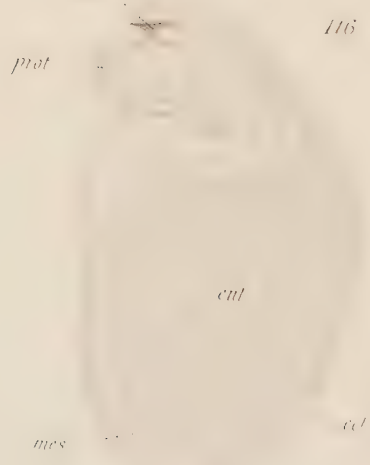

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EXPLANATION OF PLATE XIX.

Chætopterus pergamentaceus Cuvier.

FIG. 117. Side view, first cleavage; the yolk-lobe, *y*, in a very early stage.

FIG. 118. Same view, a little later.

FIG. 119. Same view, later still.

FIG. 120. Same view: yolk-lobe almost completely constricted off; nuclei of two cells nearly reconstituted.

FIG. 121. Same view: nuclei in resting stage; yolk-lobe has been resorbed.

FIG. 122. From animal pole: transition from eight to sixteen cells.

FIG. 123. From animal pole: 16-cell stage.

FIG. 124. Same view: 32-cell stage.

FIG. 125. Left side: just after 32-cell stage.

FIG. 126. From animal pole: nearly a 64-cell stage.

FIG. 127. From right side: same stage as last figure.

FIG. 128. From left side: same stage.

FIG. 129. Dorsal view: same stage.

FIG. 130. Lower pole: same stage.

FIG. 131. From vegetative pole: cross cells dividing *obliquely;* one of the polar globules within the rosette cell, *p.g.*

FIG. 132. Right side of same egg: *p.g.*, polar globule; one prototrochal cell, cp^2, *dividing again.*

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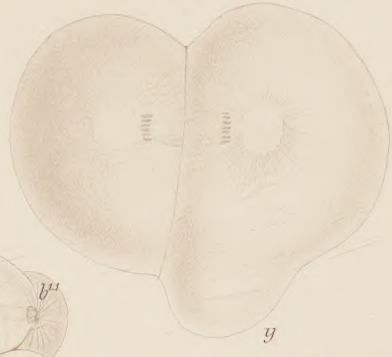

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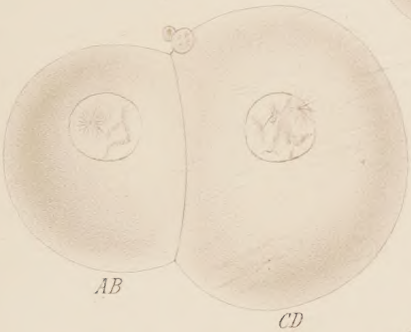

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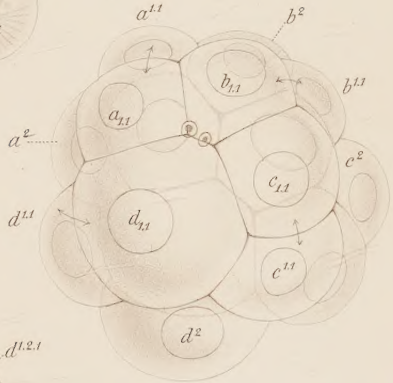

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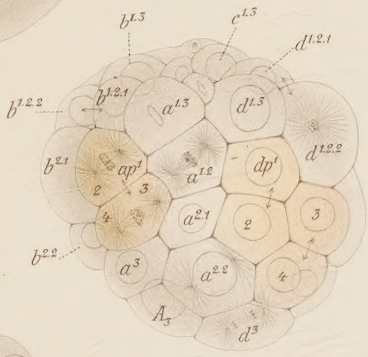

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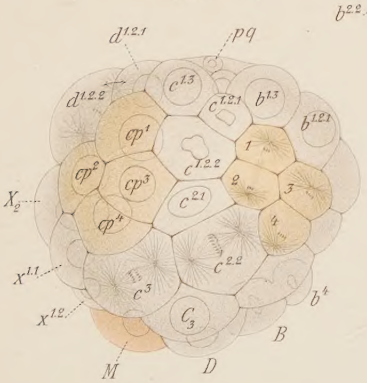

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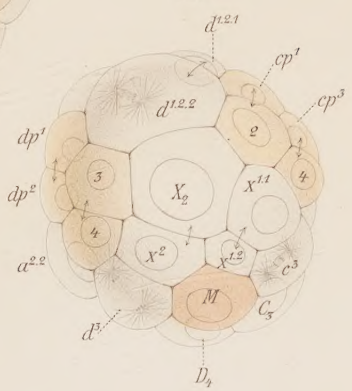

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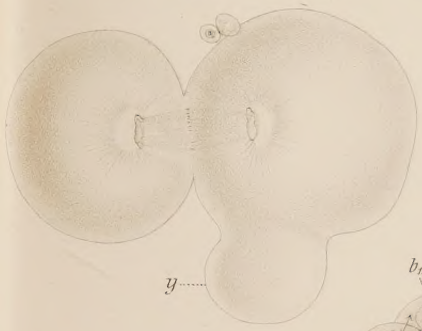

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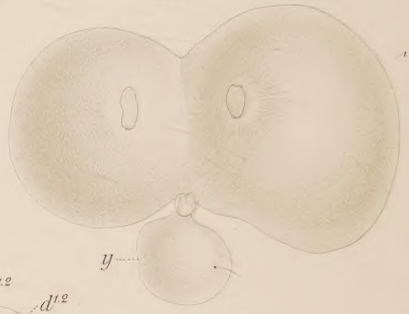

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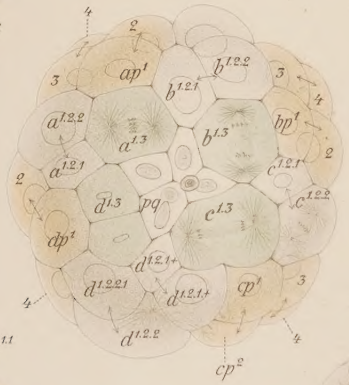

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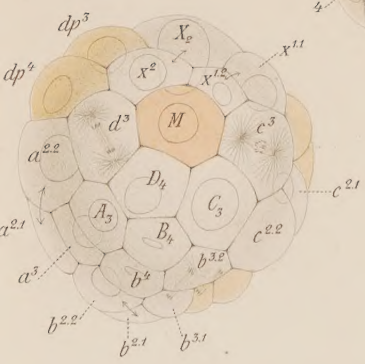

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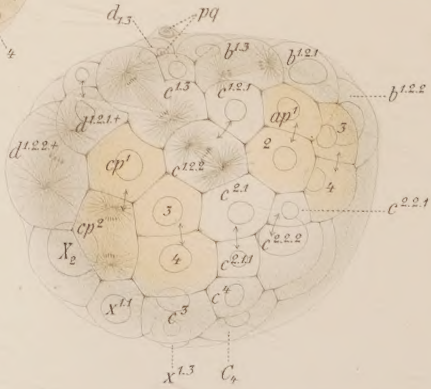

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